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Plant Parasitic Nematode Diversity in Sugarcane from Phulambri Dist.-Chhatrapati Sambhajinagar (MS.)

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Abstract: ***The nematodes were studied in Phulambri taluka. The main object of present study was to seek out soil nematodes related in sugarcane fields in Chhatrapti Sambhaji Nagar, (MS) India. Soil samples were collected from selected site Plant Parasitic nematodes were identified from sugarcane soil sample and sugarcane root sample during February 2024 to February 2025 in the laboratory of Maulana Azad College, Department of Zoology. for identification of plant parasitic nematodes stylet, esophageal, median bulb, vulva, tail etc. identified genera of nematodes, Eudorylaimus, Xiphinema, Indodorylaimus, Dorylaimus Hoplolaimus, Longidorus, species which were the serious plant pathogens of sugarcane.***
Keywords: Sugarcane, Plant parasitic nematodes, soil, root, sample etc.

I. INTRODUCTION

Sugarcane (Saccharum officinarum) belongs to the genus Saccharum, and family Poaceae. It is believed to have originatedin New Guinea and then cultivated throughout the tropical and subtropical regions of the world (Daniels and Roach, 1987). sugarcane has been recognized as an important energy source in terms of bio-ethanol production (IISR, 2015). Brazil alone accounts for 37% of the world's total sugar cane cropped area (26 million hectares) and other top 5 countries are India, Thailand, China, and Pakistan (FAO, 2018). India accounts for about 25% of the global sugar production and around 7.5% of India's rural population has engaged in sugarcane farming (Solomon, 2016).

Plant parasitic nematodes (PPNs) are microscopic, worm-like organisms that cause significant damage to a wide range of crops, including sugarcane (Saccharum officinarum). —Sugarcane is a tall growing monocotyledonous crop plant cultivated in the tropical and subtropical regions of the world for its ability to store high concentration of sucrose or sugar, in the internodes of the stem. —Sugarcane varieties are complex inter specific hybrids (Saccharum spp) cultivated to produce sugar and are derived through intensive selective breeding of species within the Saccharum genus and first line of crosses between Saccharum officinarum L. and Saccharum spontaneum L. (Cox, et al., 2000).—Worldwide, with a total production of 1076 million tons sugarcane crop occupies an area of 17 million hectares (FAO, 1996)—In India, sugarcane is cultivated in the tropical belt comprising the States of Gujarat, Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu and Kerala and the subtropical belt comprising the States of Uttar Pradesh, Haryana, Bihar, Orissa, and Punjab The planting season is from February to March in the subtropics whereas from January to February in the tropics. —In the subtropics the soil is mostly alluvial and, in the tropics, clayey, loamy, or sandy loam. —For the manufacture of white sugar, jaggery (Gur) and Khandsari in India, sugarcane is cultivated on more than 3 million hectares of land (K.C. Alexander and R. Viswanathan 1995) —Sugar industry plays a vital role in the Indian economy; sugarcane provides raw materials to over 25 industries. For over 8 million sugarcane planters it provides sustenance. Under the area of nearly 4 million hectares the production of sugarcane is about 270 million tons (Dr. Gururaj Hunsigi 2001Sugarcane is one of the major crops in Chhatrapati Sambhajinagar, Maharashtra State (India).also, one of the most important cash crops used toproduce sugar, electricity, paper, biobased chemicals, and bioethanol (Pryor et al., 2017).

The plant parasitic nematode are attack sugarcane crop. Nematodes are important pests of crop plants in both developed and developing countries of the world. Among the various species of plant parasitic nematodes, root-knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Heterodera* spp.), and lesion nematodes (*Pratylenchus* spp.) are commonly observed in sugarcane fields (Singh et al., 2020; Chawla et al., 2021). The root knot nematode (Meloidogyne) and Pratylenchus are impotent nematode pests. The Meloidogyne nematode cause galls formation of roots and reduced root growth. Pratylenchus species is migratory, moving and intra or inter cellular in the root (*Trudgil*l 2001) Plant parasitic nematodes have reported in sugarcane fields (*Williams 1969; Prasad,*

1972). plant parasitic nematodes were found associated with sugarcane *(Martin 1962)*. More than 15 thousand species of nematodes have been described the world. Of these 2200 species are identified as plant parasitic (*Goodey et al*., 1965). Plant parasitic nematodes must be stylet while non-parasitic nematodes lock of stylet for identification (*M.M.Rahman* and *I.H.Mian*)2010.

Plant parasitic nematodes are small, elongated, (0.3-2mm), pseudocoelomate eelworms of the phylum, Nematoda. When seen under microscope, plant parasitic nematodes are slender, unsegmented usually shorter than 2 mm in length with serpentine mode of locomotion. Rarely is any crop free from attack of these tiny microscopic organisms. Nematodes are minute, eel shaped worms which live in all soils. The Nematodes possess a cuticle exoskeleton, simple digestive system, and longitudinal muscles for locomotion (Siddiqi, 1985). The subtropical and tropical climate, and the extensive fibrous root system of sugarcane, provides an environment for root parasitizing nematodes. Thus, a diversity of nematode species is abundant in sugarcane fields (Spaull and Cadet, 1990). Among these, plant parasitic nematodes are major constraint in the global vegetable production. Although Phyto nematodes have wider host range and distributed Worldwide (De jin et al., 2005). They may be categorized as ectoparasitic and endoparasites. The plant may be nematodes through root, stem, leaf, flower and even fruit. Further, agricultural production is adversely affected due to pests and diseases including plant parasitic nematodes. The estimated annual yield loss of worlds major crops due to plant parasitic nematodes has been reported to the extent of 12.3% (Sasser and Freckman, 1987) and the latest estimated annual yield loss of national major crops due to plant parasitic nematodes has been reported to the extent of Rs 21,068.73 million. In economic terms, nematodes cause an estimated loss of about $ 157 billion annually to world agriculture (Abad et al., 2011). In India, Phyto nematodes are reported to cause about 10-40 %yield loss in sugarcane and could as well go as high as 50-80% in some crops such as okra, brinjal, and potatoes etc; however, losses may become still higher if Phyto nematodes are associated with other biotic and abiotic stresses in the field. Plant parasitic nematodes cause considerable loss to worldwide agriculture (Chitwood, 2003; Abad et al., 2008; Fuller et al., 2008). However, extensive information on accurate economic loss is often lacking. Large and consistent yield responses were obtained when nematode populations were reduced by soil fumigation, crop rotation, and fallowing (Stirling et al., 2001). Lesion nematode (PratylenchusZeae) is probably the most important of the community of plant parasitic nematodes that are commonly found in sugarcane fields. It occurs at high population densities in a wide range of soil types (Blair et al., 1999a,1999b) and was the predominant plant parasite at many of the sites where sugarcane responded to nematicide treatment (Blair and Stirling, 2007). At present 48 genera and 310 species of endo and ecto parasitic nematodes species have been reported to be associated with rhizosphere soil and root of various crops a including sugarcane (Cadet and Spaull, 2005). Species of five genera namely Pratylenchus, Hoplolaimus, Helicotylenchus, Tylenchorhynchus and Meloidogyne is listed as major plant parasitic nematodes with wide distribution and common occurrence in soil of India (Mehta et al., 1992). In India, there is hardly any area where the crops are not suffered from the menace caused by plant parasitic nematodes. The feeding activities of the nematodes usually result in stunting growth, reduced crop yield and occasionally plant death. Plant parasitic nematodes are considered worst enemies of a wide host range of plants. They are distributed all over the world in almost crops (Chiarappa, 1971). In India, there is hardly any area where the crops are not suffered from the menace caused by plant parasitic nematodes. The feeding activities of the nematodes usually result in stunting growth, reduced crop yield and occasionally plant death. Plant parasitic nematodes are considered worst enemies of a wide host range of plants. They are distributed all over the world in almost crops (Chiarappa, 1971).

II. HISTORY OF NEMATOLOGY IN INDIA

Nematology was only recognized as a separate branch of Agricultural Science in India 37 years ago. In chronological sequence, the history and development of nematologists in India are mentioned here.

1901 - In Tamil Nadu, Barber discovered a root nematode on tea.

Butler reported a root nematode in black pepper in Kerala in 1906.

In 1913-1919, Butler reported Urfa disease on rice in Bengal caused by Ditylenchus Augustus infestation.

Ayyar reported root-knot nematode invasion of vegetables and other crops in India in 1926 and 1933.

Dastur reported white tip disease of rice caused by Aphelenchoidesbesseyi in central India in 1934 and 1936.

In 1959, Prasad, Mathur, and Sehgal published the first grain nematode time from India.

Siddiqi discovered citrus nematodes and other plant parasitic nematodes in Aligarh soil in Uttar Pradesh in 1959.

Jones discovered the golden potato nematode in the Nilgiri Hills of Tamil Nadu in 1961.

In Kerala, Nair, Das, and Menon discovered the borer nematode Radopholussimilis on bananas in 1966.

The IARI's Division of Nematology was established in 1966 in New Delhi.

The Nematological Society of India was established in 1969.

The Nematological Society of India began publishing its semi-annual magazine, "Indian Journal of Nematology," in 1971.
The All India Coordinated Research Project (AICRP) on crop nematodes and their control began in 1977, with the project coordinator based at IARI in New Delhi.

A. *Life Cycle of PPN's*

Egg: Female nematodes lay eggs in or outside of the plant, either singly or in masses. The eggs hatch into larvae (juveniles) that look like adults. Juvenile stages: The first four stages are the juvenile stages, J-1 to J-4. The J2 stage is the first stage that hatches from the egg (Jones, J. T., et al. (2013)).
Penetration & Migration: parasitic nematodes penetrate the plant tissue using various structures such as stylet & secretions that aid in piercing the cell wall. They migrate through the plant root system seeking suitable feeding sites causing physical damage to root cells (Abad, P., et al. (2008)).
Feeding: Once a suitable feeding site is found, nematodes use their stylet to puncture the plant cells & feed on contents including cell sap, nutrients, & even some cell organelles.
In some cases, nematodes induce the formation of specialized feeding structures such as root knot (galls or balls formation), nematode pores (lesions), or cysts which contain eggs.
Secretion of effector molecules: Parasitic nematodes secrete various effector molecules into the plant tissue which includes enzymes & proteins which manipulates host cell physiology & suppresses plant defense responses (Sikora, R. A., et al. (2005)). These effector molecules may alter the hormone signaling pathway & suppress immune responses and induce the formation of specialized feeding structure. Cell wall modification: Nematodes can induce changes in the composition of structure of plant cell wall leading to alterations in cell wall & composition. Vascular damage: Nematode feeding and migration can disrupt the plant vascular system, nutrient uptake & transportation.

III. MORPHOLOGY AND ANATOMY OF NEMATODES

Nematodes have the following body parts:

1) Cuticle: The outermost protective covering of the nematode body, made of collagen and other proteins that give it elasticity. It is a non-cellular layer that plays an important role in providing structural support and preventing desiccation.
2) Hypodermis: Beneath the cuticle lies the hypodermis, which is a layer of cells where nutrition storage (secretion of cuticle) takes place.
3) Musculature: Nematodes possess a simple longitudinal muscle system. The muscle cells are arranged in four quadrants: two dorsal and two ventral's.
4) Pseudocoelom: Nematodes have a fluid-filled body cavity that provides space for internal organs and facilitates the movement of nutrients and gases.
5) Digestive system: The digestive system of nematodes is relatively simple and includes a mouth, pharynx, intestine, and anus. The pharynx contains muscles that allow nematodes to feed by sucking in food.
6) Reproductive system: Nematodes are diecious (sexes are separate) and hermaphrodite. Males have a specialized copulatory structure, while the female produces eggs.
7) Sense organs: Nematodes have various sensory structures, including papillae and amphids. Papillae are small finger-like projections that may be involved in touch and chemoreception. Amphids are specialized sensory organs located near the anterior end, often associated with chemo-sensation.
8) Excretory system: Nematodes possess a simple excretory system known as the Sas excretory gland or canal. The excretory system in nematodes helps to regulate the osmotic balance and remove waste.

IV. MATERIALS AND METHODS

A. *Soil Sampling*

The Sugarcane fields were randomly selected for soil sampling from Phulambri village in Chhatrapati sambhajinagar District. Total Thirty-eight soil samples were collected from thirteen sites. Of the diameter 1.9cm, from sugarcane plant to a depth of 15-20cm. The collected soil samples were sealed in a polyethylene bag and kept away from sun. The samples were properly tagged and carry to the research lab at Department of zoology, Maulana Azad College Chhatrapati sambhajinagar (M.S.) for the extraction, processing, and identification of nematodes.

B. Nematode Extraction

Cobb's Decanting and Sieving

Materials:

1. 20 Mesh sieve
2. 60 Mesh sieve
3. 150 Mesh sieve
4. 325 Mesh sieve
5. 1 Bucket
6. 250 ml beaker
7. 200 ml beaker
8. Running tap water

Procedure

Major the soil in the glass beaker (200 cc) in bucket.

Mix in the soil sample minimum 2 Liter water. Stirring with hand and remove the rocks and roots.

20 mesh sieves, 60 mesh sieve, 150 mesh sieve and 325 mesh sieves arrange.

Pour the soil sample in sieve alternatively back-to-back wash under the tap water.

One by one sieve back-to-back wash and clear water sample collect the 250 ml beaker. In case sample in more amount, they farther diluted in 325 mesh sieves. The collected samples soil sample were taken for nematode extraction By Baermann funnel technique.100ml soil samples placed into a beaker and closes the beaker mouth by tissue paper. And keep it for24 hours. Sample were removed from beaker, and the nematode suspension was poured into a wash bottle and allowed to settle Once the supernatant was removed, and the remaining suspension that contain nematodes, then this suspension was poured into the nematode investigating dish for nematode investigation andexamined under the stereo and light microscope.

Use the Microscope for Observation.

1. Stereo microscope use the Nematode population given sample.
2. Compound microscope observation internal and external structure. 10x, 40x, 100x.

C. Fixation Of Nematodes

Different types of fixatives and solutions are required for preparing temporary and permanent whole Mount of the nematodes. T. A. F. Was used for as a fixative solution for permanent mounting of nematodes.

T. A. F

Chemical formalin (=37% formaldehyde) 7.6 ml

Tri-ethylamine 2ml

Distilled water 90.4 ml

The T. A. F Fixative is heated to about 50-60 C and directly poured on the Nematode Specimen and Fixative Specimen is kept for about 48 hr. before making permanent slides, fixing inT.A. F method keep the seen nematodes in the T.A.F. for 6 days.

D. Preservation of Neamtodes

Seinhorst 1 (preserve nematodes for 4 days) (2 ml)

Seinhorst2(preserve nematodes for 6 days)(2ml)

E. Permanent Slides Preparation

Materials Required

Metal Rod

Slides

Cover Glass

Metal plate

Glycerin

Fixed Nematode

Paraffin Wax.

The metal rod was heated and dipped in wax and then metal rod was placed on the slides and paraffin wax ring was put on slide, A drop at glycerin was added in the parrafin wax ring fixed Nematode were then placed in the glycerin drop, after this the cover glass was carefully placed on the parrafin ring the slide was then placed on the metal plate the had a spirit lamp below it due to the heat the parrafin wax ring melts and it was then allowed it cool the wax forms a seal and the permanent slides were made, the slide was then placed on the melts and it was then allowed to cool the wax forms a seal and the permanent slide were made in that case use the nail polish to seal it

F. Nematodes Identification

Observed different structures stylet, esophageal, median bulb, vulva, tail etc and finally identified with the help of pictorial key to genera of plant parasitic nematodes (*Mai and Lyon 1975).*

V. RESULT AND DISCUSSION

Identified 6 genera of plant parasitic nematodes with their systematic position.

Table 1

Sr.no	Genera Of Nematode	Common Name Of Nematodes	Identifying Character Of Genera	Classification
1.	Hoplolaimus	Lance Nematodes.	Spear is massive with strongly developed basal knobs, tail is rounded.	Phylum:Nematoda Class:Secernentea Order:TylenchidaFamily: Tylenchidae
2.	Longidorus	Needle Nematode	Very long, slender body - Extremely long stylet - Esophageal glands extend far back - Females with elongated tails	Phylum:Nematoda Class:Secernentea Order:Dorylaimida Family:Longidoridae
3.	Eudorylaimus	Dorylaimids	Medium-sized body - Stylet relatively short - Lip region often rounded - Tail varies in shape	Phylum:Nematoda Class:Secernentea OrderDorylaimida Family:Eudorylaimidae
4.	Xiphinema	Dagger Nematodes	Medium to large body size - long stylet, often curved - Distinctive lip region with well-developed framework - Females with characteristic tail shape	Phylum:Nematoda Class:Secernentea Order:Dorylaimida Family:Xiphinemidae
5.	Indodorylaimus	Roundworms	Similar to Dorylaimus but with some differences in stylet and tail morphology	Phylum: Nematoda Class: Secernentea Order: Dorylaimida Family: Dorylaimidae
6.	Dorylaimus	Dorylaimid	Medium sized body - Stylet relatively short - Lip region rounded or slightly flattened - Tail conical or rounded	Phylum: Nematoda Class: Secernentea Order: Dorylaimida Family: Dorylaimidae

VI. DISCUSSION

Six genera of plant parasitic nematodes were identified. Identified of pant parasitic nematodes with their systematicposition and identified the nematode. The different structures such as stylet, basal knobs, and vulva and tail etc, were observed. The present study revealed some interesting facts about the nematode diversity of the Chhatrapati sambhajinagar district. Around the sugarcane root zone we are identified Six genera which are belongs to genus Dorylaimus, Indodorylaimus, Xiphinema, Eudorylaimus, Longidorus, and Hoplolaimus. Out of these six genera the genus Dorylaimus shows high frequency and dominance than the other genera from all selected fields.

Monthly diversity of nematodes from Phulambritaluka of Chhatrapati. Sambhaji Nagar district during January-2024- December 2024.

Table: 2

Months	Name of the Neamtodes	No. of Nematodes
Jan	Eudorylaimus	21
feb	Dorylaimus	13
Mar	Xiphinema	37
Apr	Hoplolaimus	10
May	Dorylaimus	42
June	Dorylaimus	13
July	Hoplolaimus	09
Aug	Dorylaimus	47
Sept	Indodorylaimus	43
Oct	Hoplolaimus	33
Nov	Longidorus	29
Dec	Dorylaimus	38
		335

VII. CONCLUSION

The current study provided some fascinating information regarding the diversity of plant parasitic nematodes in the Chhatrapati sambhajinagar. The higher nematode diversity was recorded of genus Dorylaimus from all selected fields. Most of the nematode diversity was recorded in February, August, and April month from Phulambritaluka. Six genera of plant parasitic nematodes identified in soil samples and root samples. For identification of plant parasitic nematodes stylet, vulva, basal bulbs, and tail type are very useful.This subject needs any further investigation. This study will be more helpful to the students, Research scholars and Nematologists.

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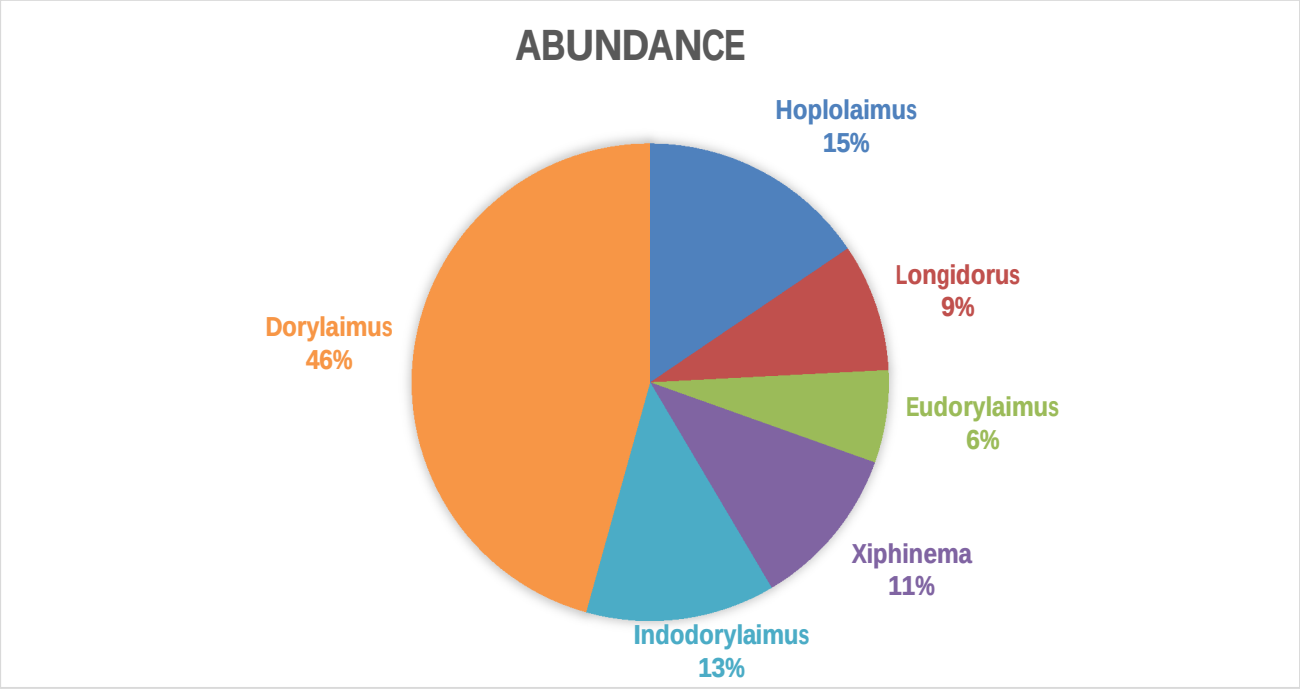

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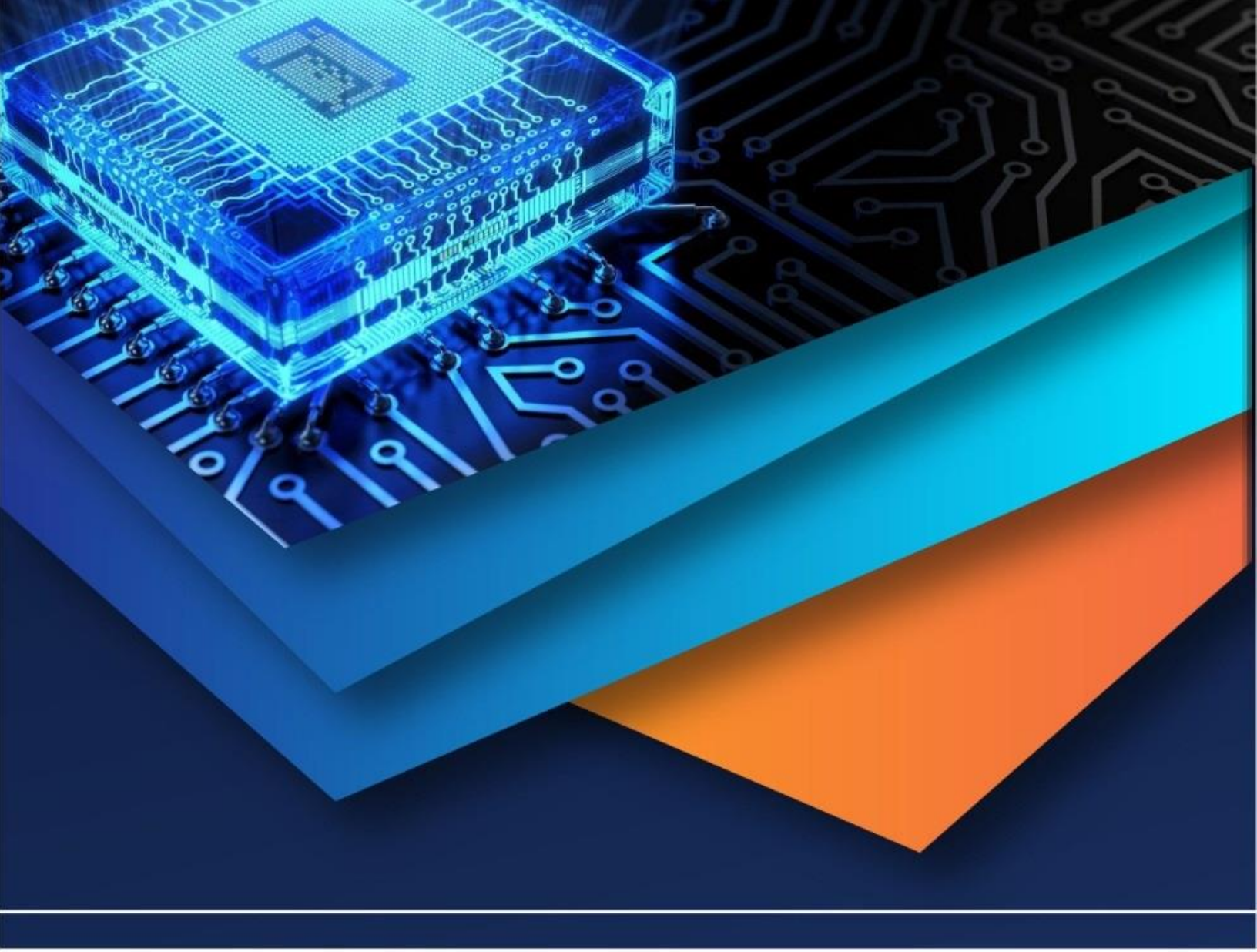

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