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Effect of Different Concentration of Fungicide Mancozeb on Protein Concentration and Alpha Amylase Activity of *Eleusine coracana.* (L.) Gaertn.

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Abstract: ***Experiments were carried out to study the effect of fungicide Mancozeb on seed germination, seedlings growth, chlorophyll content, protein concentration and alpha amylase activity. The experiments were conducted under a completely randomised design, consisting of 8 treatments in a 2×4 fractorial system [2 seed variety × 4 Mancozeb Concentration : 0.2%, 0.3%, 0.4%, 0.0% (control) in 3 replicates each consisting of 20 Ragi seeds.. The Ragi variety used was INDAF 7 and KMR 301. In KMR 301, the highest percentage of germination, high chlorophyll content, high protein concentration and high alpha amylase activity was recorded in seeds treated with 0.4% concentration of Mancozeb and lowest was recorded in control. In INDAF 7, the highest percentage of germination, high chlorophyll content, high protein concentration and high alpha amylase activity was recorded in Control and lowest was recorded in seeds treated with fungicide concentrations (0.2%, 0.3%, 0.4%).***
Keywords: Fungicide, Mancozeb, Germination, Vigour, Chlorophyll, Protein, alpha amylase.

I. INTRODUCTION

Ragi (*Eleusine coracana (L)* Gaertn.) commonly known as Finger millet, mandua, nagli, kapai and madua is widely cultivated in India. Karnataka state has the highest area of about 1.0 m.ha (60% of the total), followed by Maharashtra, Odisha, Tamil Nadu, Andhra Pradesh and Uttarakhand. For its rich nutritive value in terms of 8-10 times more calcium than that of rice and wheat, high quality protein, plenty of minerals, dietary fiber, phytochemicals and vitamins it has become popular in human diet. *Magnaporthe grisea* (Hebert) Barr. (Anamorph: *Pyricularia grisea*) is the most important constraint in finger millet production [1] . Finger millet grains contain 13.24% moisture, 7.6% protein, 74.36% carbohydrate, 74.36% carbon, 1.52% dietary fiber, 2.35% minerals, 1.35% fat and energy 341.6 cal/100g [2].

Ragi is also known for several health benefits such as anti-diabetic, anti-tumerogenic, atherosclerogenic effects, antioxidant, which are mainly attributed due to its polyphenol and dietary fiber contents. Being indigenous minor millet it is used in the preparation of various foods both in natural and malted forms. Grains of this millet are converted into flours for preparation of products like porridge, puddings, pancakes, biscuits, roti, bread, noodles, and other snacks. Besides this, Ragi is also used as a nourishing food for infants when malted and is regarded as wholesome food for diabetic's patients.

The association between fungi and seeds can severely affect seed quality, reducing germination, vigour, seedling emergence and productive potential. It is not always possible to obtain seed lots 100% guaranteed free of pathogens. It is also not possible to ensure that the sown soil or substrate will be clear of fungi. Therefore, seed treatment is advisable in most cases, especially for vegetable hybrid seeds, because treatment cost is very low compared to the high price this type of seeds carries [3]. Seed treatment has been efficient in preventing plant disease outbreaks caused by pathogenic agents in seeds, particularly fungal agents. Chemical seed treatment aims to eradicate these pathogens and/or to protect against soil pathogens, especially during germination. Furthermore, seed treatment can help reduce the volume of fungicides needed to control the diseases. Seed treatment can eliminate the need of foliar use of fungicide products in field crops [4].

Fungicides represent one of the most effective and integrative methods of controlling disease by acting against phytopathogenic fungus in agriculture.

However, the toxicity and the pollution generated by fungicides cannot be neglected and their toxic effect on seeds depends on their distribution, concentration, persistence and metabolism of its active ingredients [5].

Mancozeb is an organomettalic polymeric complex of manganese zinc ethylene bisdithiocarbamate fungicide. It is extensively used in agriculture to protect many fruits, vegetables, field crops and seeds against large spectrum of fungal diseases [6].

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II. MATERIALS AND METHODS

A. *Collection of Samples*

The study was conducted at Department of Botany, Yuvaraja's College Mysore, Mysuru. This was carried out for a period of four months. 2 different varieties of finger millet KMR 301 and INDAF-7 were collected from VC farm Mandya.

B. *Seed treatment*

Ragi seeds were washed with distilled water and surface sterilized by mercuric chloride for 15min and soaked in different concentrations of fungicide (Mancozeb) solution. (control, 0.2%, 0.3%, 0.4%) 24 hrs later they were kept for germination in the germination papers. 7 days old seedlings were selected for protein estimation and 14 days old were selected for chlorophyll estimation.

C. *Seed Germination percentage*

Germination refers to initial appearance of radical by visual observation. The varieties of treated Ragi seeds were placed on germination paper and rolled and maintained in moist condition. The number of seeds germinated was counted on 7^{Th} and 14^{th} day of germination and total germination percentage was worked out. It was calculated by using the following formula according to ISTA, 2003 [7].

Germination percentage = (no of seeds germinated ÷ Total no of seeds sown) × 100

D. *Seedling Vigour Index*

The Seedling Vigour Index was calculated by using the formula proposed by Abdul Bak and Anderson (1973) and expressed in whole number [8].

Seedling Vigour Index (SVI) = % Germination X Mean seedling length.

E. *Estimation of chlorophyll*

The chlorophyll contents viz. chlorophyll-a and chlorophyll-b and total chlorophyll were estimated as per the method of Arnon (1949) [9].

Chlorophyll calculation

$$\text{mg chlorophyll a/g tissue} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll b/g tissue} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

and

$$\text{mg total chlorophyll/g tissue} = 20.2\,(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$$

Where ,

A = absorbance at specific wavelengths

V = final volume of chlorophyll extract in 80% acetone and

W = fresh weight of tissue extracted

F. *Protein estimation*

Sample preparation for protein estimation: Protein estimation was carried out by Lowry's method [10].

G. *Estimation of Enzyme*

Preparation of samples for amylases: 100mg seedlings Sample was placed in mortor with 1 ml 0.02M phosphate buffer and the extract was spun at low speed (10.000 rpm for 20min). The supernatant homogenate was used for enzyme assay.

α - AMYLASE : Amylase content was determined as per the procedure described by Peter Bernfield (1955) [11].

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III. RESULTS

(KMR-301 variety)

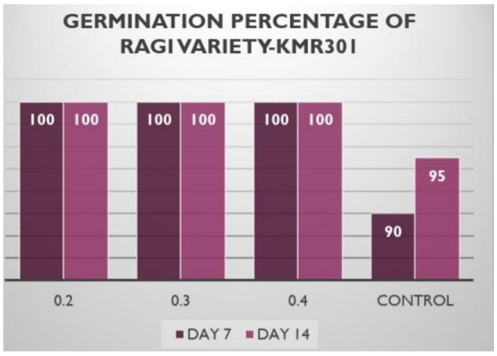
Table 1 : Morphological growth parameters of KMR-301 in 7th and 14th day seedlings.

Morphology parameters	*KMR 301 treated with different concentration of fungicide*							
	0.2%		0.3%		0.4%		Control	
	7th Day	14th Day	7th Day	14th Day	7th Day	14y Day	7th Day	14th Day
Mean root length in cm	5.80	6.325	5.6	6.12	6.085	6.685	4.95	5.59
Mean shoot length in cm	3.23	3.93	3.32	4.01	3.40	4.12	2.71	3.41
Number of leaves	1.42	1.8	1.6	1.8	1.7	2	1.42	1.7
Number of roots	1	1	1	1	1	1	1	1
Weight in grams	0.6	0.69	0.59	0.67	0.61	0.711	0.54	0.62

Table 2 : Germination percentage and vigour index in 7th and 14th day seedlings of KMR-301

SL. No.	*Parameters*	*Percentage of germination*		*Vigour index*	
		7th Day	14th Day	7th Day	14th Day
1	0.2%	100	100	904	1025.5
2	0.3%	100	100	892	1013
3	0.4%	100	100	948.5	1080.5
4	CONTROL	90	95	689.4	855

Graph 1 : Graph showing Germination percentage and vigour index in 7th and 14th day seedlings.



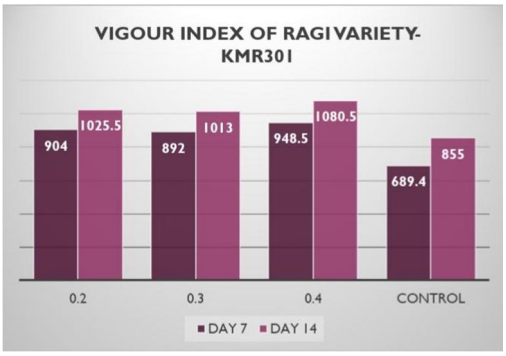


Table 3 : Chlorophyll estimation in leaves of 14th day old seedlings of KMR-301

Fungicide concentration	*Chlorophyll a*	*Chlorophyll b*	*Total chlorophyll*
	In (mg/g)		
0.2%	0.029	0.045	0.075
0.3%	0.0090	0.088	0.097
0.4%	0.011	0.092	0.103
Control	0.0010	0.054	0.074

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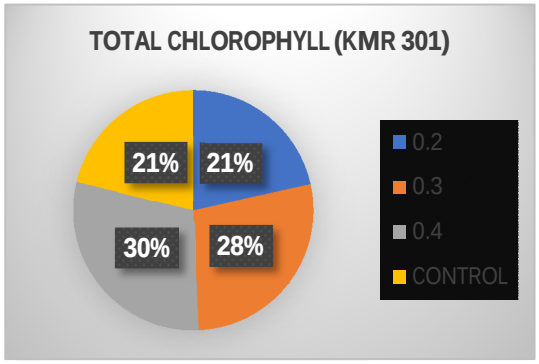
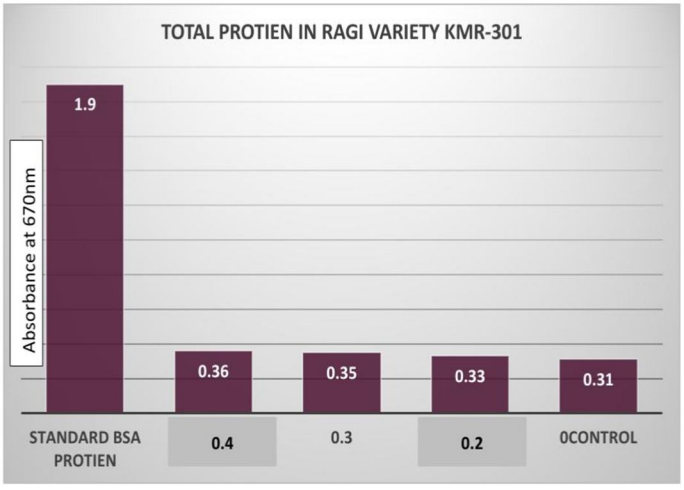


Graph 2 : Graph showing Total chlorophyll

Table 4 : Estimation of Protien concentration in 14th Day seedlings of KMR-301

Total protein present in 1ml (100 µg/ml) of Enzyme		
SL NO	Parameters	Absorbance at 670nm
1	Standard- BSA Protien	1.9
2	0.2%	0.33
3	0.3%	0.35
4	0.4%	0.36
5	Control	0.31



Graph 3 : Graph showing protein concentration

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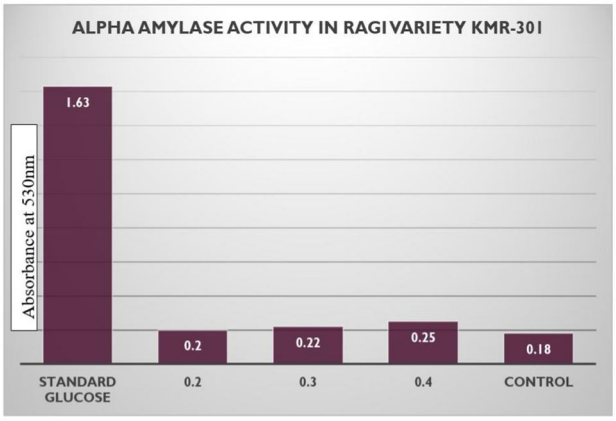
Table 5 : Estimation of total alpha amylase activity in 14th Day seedlings of KMR-301

ALPHA- Amylase activity in 1ml (100 µg/ml) of Enzyme

SL NO	Parameters	Absorbance at 530nm
1	Standard- glucose	1.63
2	0.2%	0.2
3	0.3%	0.22
4	0.4%	0.25
5	Control	0.18



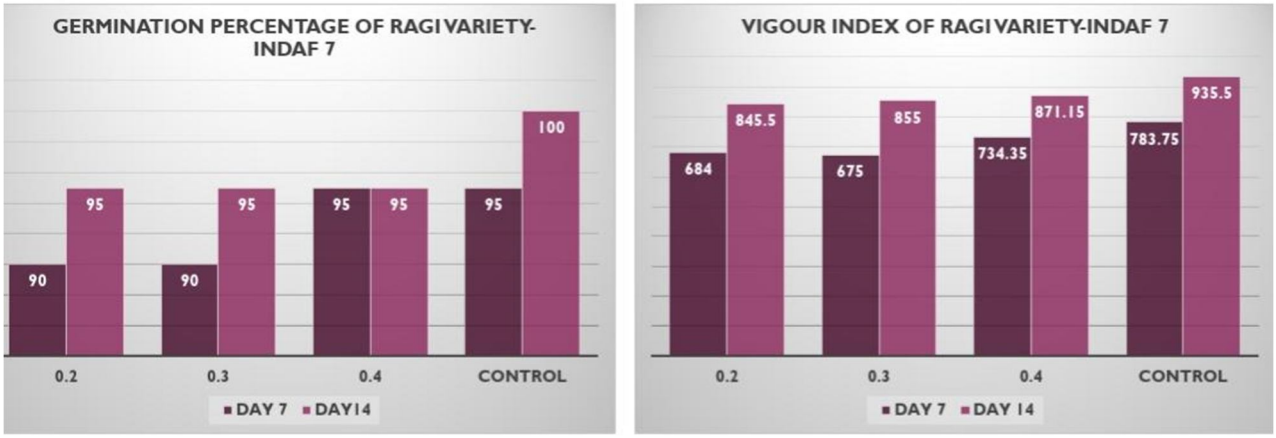
Graph 4 : Graph showing Absorbance of Alpha amylase. INDAF-7

Table 6 : Morphological growth parameters in 7th and 14th day seedlings of INDAF-7

Morphology Parameters	*INDAF-7 treated with different concentration of fungicide*							
	0.2%		0.3%		0.4%		Control	
	7th Day	14th Day	7th Day	14th Day	7th Day	14y Day	7th Day	14th Day
Mean root length in cm	5.1	5.8	4.8	5.23	5.1	5.6	5.31	6.05
Mean shoot length in cm	2.5	3.1	2.7	3.77	2.63	3.57	2.94	3.305
Number of leaves	1.21	1.42	1.1	1.7	1.3	1.6	1.4	1.8
Number of roots	1	1	1	1	1	1	1	1
Weight in grams	0.501	0.57	0.54	0.57	0.55	0.59	0.6	0.68

Table 7 : Germination percentage and vigour index in 7th and 14th day seedlings of INDAF-7

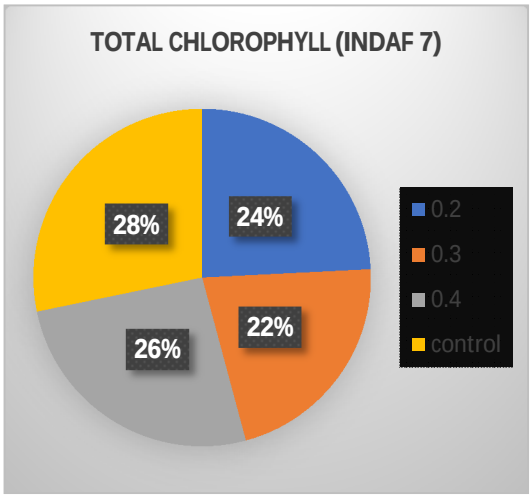
SL. No.	*Fungicide concentration*	*Percentage of germination*		*Vigour index*	
		7th Day	14th Day	7th Day	14th Day
1	0.2%	90	95	684	845.5
2	0.3%	90	95	675	855
3	0.4%	95	95	734.35	871.15
4	Control	95	100	783.75	935.5



Graph 5 : Graph showing Germination percentage and vigour index in 7th and 14th day seedlings.

Table 8 : Chlorophyll estimation in leaves of 14th day old seedlings of INDAF-7

Fungicide concentration	*Chlorophyll a*	*Chlorophyll b*	*Total chlorophyll*
0.2%	0.032	0.051	0.084
0.3%	0.029	0.045	0.075
0.4%	0.029	0.061	0.090
Control	0.030	0.067	0.098

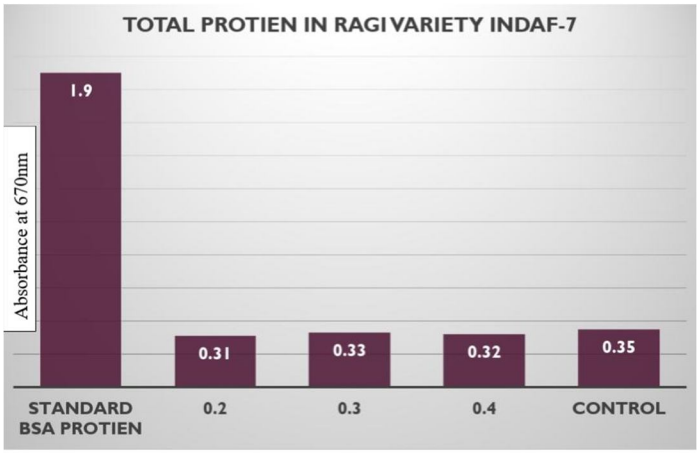


Graph 6 : Graph showing Total chlorophyll

Table 9 : Estimation of Protien concentration in 14th Day seedlings of INDAF 7

INDAF-7 protein present in 1ml (100 µg/ml) of Sample

SL NO	Parameters	Absorbance at 670nm
1	Standard-BSA Protien	1.9
2	0.2%	0.31
3	0.3%	0.33
4	0.4%	0.32
5	Control	0.35

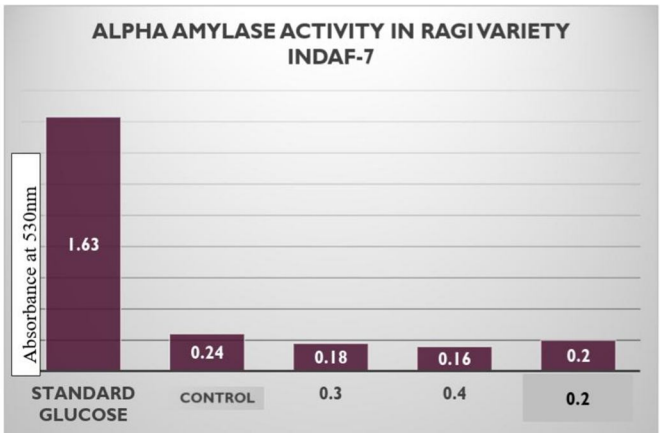


Graph 7 : Graph showing protein concentration

Table 10 : Estimation of total alpha amylase activity in 14th Day seedlings of INDAF 7

ALPHA- Amylase activity in 1ml (100 µg/ml) of Enzyme

SL NO	Parameters	Absorbance at 530nm
1	Standard- glucose	1.63
2	0.2%	0.2
3	0.3%	0.18
4	0.4%	0.16
5	Control	0.24



Graph 8 : Graph showing Absorbance of Alpha amylase.

IV. DISCUSSION

IN KMR-301 variety : The effect of Mancozeb on Ragi seeds was assessed. It was noticed that at 0.4% concentration treatment of Mancozeb gave high possitive affect when compared to Control, because Mancozeb fungicide protected seeds from fungus causing organisms and resulted in good growth of the seedlings by reducing the destruction of seeds. In control as there was no protection for fungus led to the decrease in germination rate, seedlings growth, Chlorophyll content, protein and aplha amylase activity.

The Morphological growth parameters like mean root length, mean shoot length, mean weight of seedlings, nunber of leaves and number of roots, Germination percentage, vigour index were studied on 7th and 14th Day. And total chlorophyll content, Protein concentration, alpha amylase activity was studied. Highest and positive result was observed in seeds treatment with 0.4% fungicide concentration and decreased growth rate was observed in Control (untreated). In the 0.4% concentration at 14th day mean root length was 6.685 cm, mean shoot length was 4.12 cm, weight of seedlings was 0.711 g, number of leaves was 2, and there was no difference in the number of roots (Table 1).No difference was observed in germination percentage of seeds treated with different concentrations(0.2, 0.3, 0.4) it was 100% and low germination percentage of 95% was observed in Control. Highest vigour index at 14th day was 1080.5 observed in 0.4% concentration (Table 2). Highest Total Chlorophyll content was observed in 0.4% concentration is 0.103 and low content of chlorophyll was observed in control is 0.074 (Table 3). High protein concentration was observed in 0.4% concentration is 0.34 and it was low in Control is 0.31 (Table 4). High activity of alpha amylase was observed in 0.4% concentration is 0.25, and low in control is 0.18 (Table 5).

It can be reported that fungi incidence decreased in seeds with increasing fungicide doses, In general the fungicide Mancozeb promotes a reduction of fungus in Ragi variety KMR-301 and thus giving positive affect o growth parameters of seedlings [12].

In INDAF-7 variety : In this variety it was noticed that Control (untreated) gave high positive affect when compared to all other concentration treated with fungicide.

The Morphological growth parameters like mean root length, mean shoot length, mean weight of seedlings, number of leaves and number of roots, Germination percentage, vigour index were studied on 7th and 14th Day. And total chlorophyll content, Protein concentration, alpha amylase activity was studied. Highest and positive result was observed in Control (untreated) and it was low in fungicide treated concentrations (0.2%, 0.3%, 0.4%). In the Control at 14th day mean root length was 6.05 cm, mean shoot length was 3.305 cm, weight of seedlings was 0.68 g, number of leaves was 1.8 and there was no difference in the number of roots (Table 6). No difference was observed in germination percentage of seeds treated with different concentrations(0.2, 0.3, 0.4) it was 95% and high germination percentage of 100% was observed in Control. Highest vigour index at 14th day was 935.5 observed in Control (Table 7). Highest Total Chlorophyll content was observed in Control is 0.098 (Table 8). High protein concentration was observed in Control is 0.35 (Table 9). High activity of alpha amylase was observed in control is 0.24 (Table 10). It can be reported that Increased Mancozeb concentrations caused reduction in all the studied parameters of seedlings.

Fungicides is phytotoxicity, or a toxic effect on beneficial plants. It's important to use the right type of fungicide on the right plant at the right time, or you may have problems. For instance, the fungicide azoxystrobin, frequently used on grapes, can kill some apple varieties, while trifloxystrobin is harmful to certain grape cultivars but not others. Some fungicides are growth specific, such as triazole + QoI fungicides that can't be applied to soybeans later than a growth stage known as R5.

Germination percentage of Mancozeb fungicide treated seed was decreased with the increase in concentration of fungicide Mancozeb. Other studies also reported that more fungicide produces negative interference and abiotic stress in germination of seeds and decreased it drastically in the treated sets ([13] [14] [19]).

The results of present study demonstrated that shoot and root length was decreased with increasing concentration of fungicide [15]. Seedling vigour index was decreased at almost all the concentration of fungicide as compared to control in the present study. But the results of our present study were contrary to Morales et al. (2012) [16] who observed higher vigour of rice seeds treated with Carboxin and Thiram fungicides. Chlorophyll content were also reduced in the study as increased in concentration of Mancozeb fungicide and the results of our study were similar to the effect of tricyclazole on Maize seeds (Avinash 2012) ([17] [19])

Mancozeb is a systemic fungicides which are based on sterol biosynthesis inhibitor are closely related to plant growth regulators the use of which at higher than labeled rates shorten the internodes which may lead to slow-shoot and root growth ([18][19]).

V. CONCLUSION

From the present study it was concluded that in Ragi variety KMR-301 Mancozeb treatment at 0.4% concentration favours growth of seedlings. In general the fungicide Mancozeb promotes a reduction of fungus in Ragi seeds and thus giving positive affect on growth parameters of seedlings when compared to Control.

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In Ragi variety INDAF-7 Mancozeb treatment negatively effected the seedlings growth, chlorophyll content, protein and alpha activity and positive response was recorded in Control. However, the toxicity and the pollution generated by fungicides cannot be neglected. The review examines toxic effect of a fungicide on seeds depends on its distribution, persistence, metabolism, its active form, and its concentration. Some fungicide interfere with the metabolic pathways of plants and lead to destruction of seed quality

It is concluded that, Affect of fungicides depends on the variety, abiotic stress, distribution, persistence, metabolism, its active form and its concentration. It may show positive effect in some variety with increasing the fungicide concentration and same can show negative effect or become toxic to plants with increased fungicide concentrations. Testing should be carried out before applying to field condition

From the study, if KMR-301 is selected it should be treated with 0.4% concentration of Mancozeb for better results. If INDAF-7 is selected it is suggested to grow without treating the seeds with Mancozeb fungicide.

The findings of the experiment can be used to educate the farmers hence it is applicable to field condition.

VI. PHOTOGRAPHY

Total Chlorophyll Estimation in 14th day seedlings.

7th Day old seedlings of KMR 301 variety.

7th Day old seedlings of INDAF 7 variety.

14th Day old seedlings of KMR 301 variety.

14th Day old seedlings of INDAF 7 variety.

Total protein in 7th day seedlings of Ragi variety- KMR 301

Total protein in 7th day seedlings of Ragi variety- INDAF 7

Alpha amylase activity in 14th day of Ragi variety KMR-301

Alpha amylase activity in 14th day of Ragi variety INDAF-7

VII. ACKNOWLEDGEMENT

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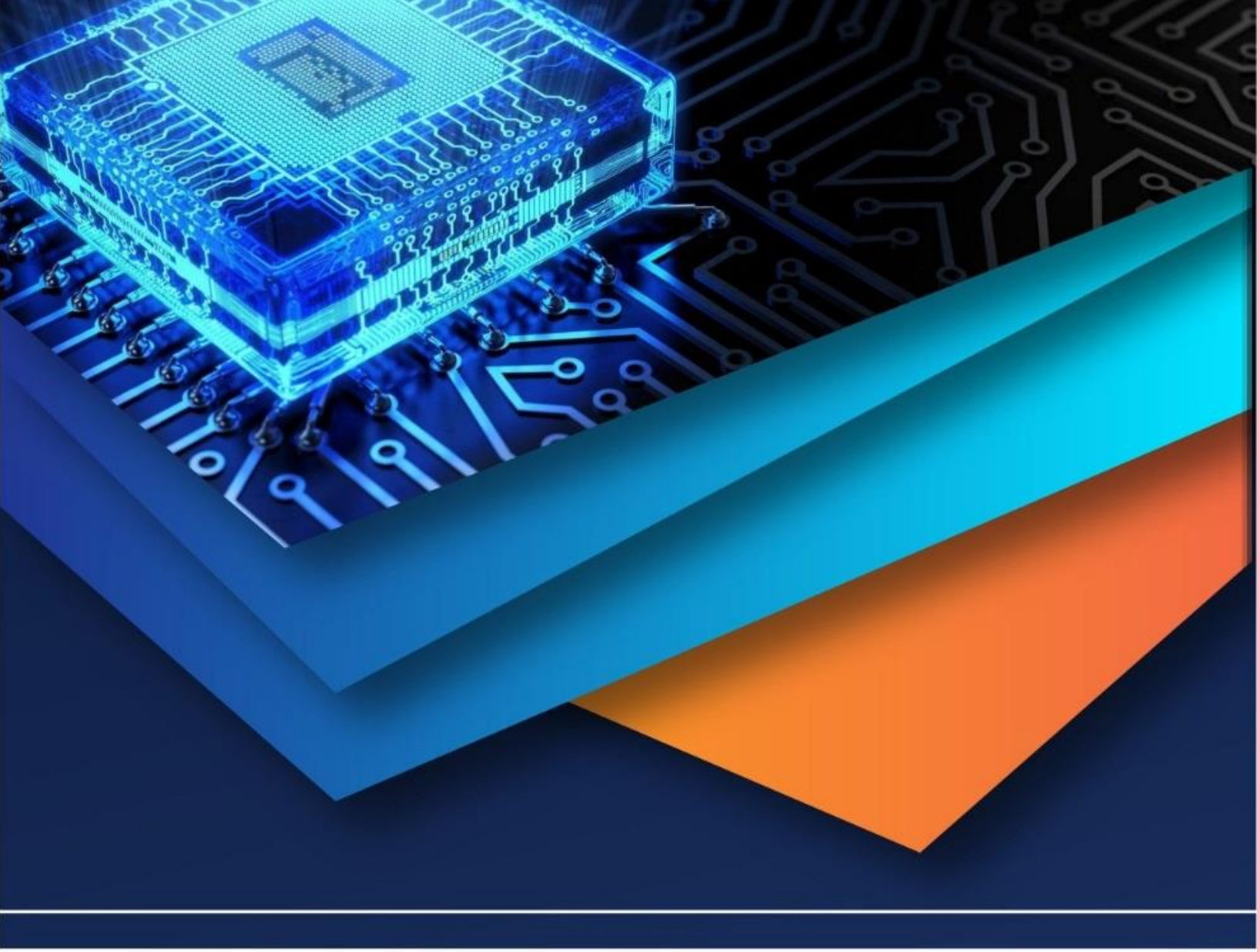

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