



RESEARCH ARTICLE

**CARBENDAZIM INDUCED ALTERATIONS IN MORPHOLOGICAL RESPONSES,
CHLOROPHYLL, AND PHENOLIC CONTENTS OF RICE CULTIVARS**

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ABSTRACT

In the present study impact of carbendazium on seed germination, seedling length, electrical conductivity, vigour Index, Chlorophyll a, Chlorophyll b, total Chlorophyll, and phenolic contents of rice cultivars were studied. The study was carried out for 14 days after soaking the seeds in different concentration of carbendazium and a control was maintained. The results showed increasing trend in germination percentage, conductance and phenolic contents. The germination parameters like root length, shoot length, vigour index increased upto 9mg and at higher concentration i.e., 12mg it declined. In Jyothi and IR cultivar the chlorophyll content was found to be increased upto 9mg and there after it declined. But in Jaya Cultivar maximum chlorophyll content was recorded at 12mg concentration. Jyothi variety was less affected in terms of root length, shoot length and vigour index as compared to IR and Jaya cultivar. Thus it exhibits a degree of tolerance to fungicide.

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INTRODUCTION

Fungicide application can improve the genetic potential & crop productivity yield (1). In general most of the fungicides acts by inhibiting the energy metabolism, blocking biosynthesis or altering cell membranes of fungus (2). Carbendazium (Benzimidazoles) a systemic fungicide with curative & protection action, extensively used in agriculture, inhibit the development of germinal tube, formation of the apresora and growth of nucleus (3). Seed treatment with carbendazium 2g/kg is most effective method for the control of blast disease .Foliar application is recommended for the management of sheath blight disease (4).Unplanned and repeated use of fungicides showed phytotoxicity. They alter or inhibit physiological and metabolic activities in plants. They interfere with the formation of micro tubules by binding to protein sub unit-tublin which is involved in several cellular processes including mitosis and maintenance of cell shape (5) Carbendazim produced chromosomal aberration in somatic and germ cell of pearl millet and sunflower (Harichand *et al* 1991).It is known that fungicides induces both metabolic and biochemical changes that will affect the growth and development of plants. Therefore the present work was undertaken to evaluate the

impact of phytotoxicity of carbendizium fungicide on vigour index, percentage of seed germination, electrical conductivity, chlorophyll a, chlorophyll b, Total chlorophyll & phenolic contents of rice seedlings or paddy cultivars.

MATERIALS AND METHODS

Paddy seeds were procured from VC farm, University of Agriculture science, Mandya, Karnataka. Seeds were surface sterilized with 0.1% mercuric chloride for 10minutes and repeatedly washed with distilled water for 4-5 times to remove the excess chloride .Seeds of uniform size were selected and soaked for 24 hours in distilled water (control)and with different concentrations (mg/g)of Carbendazim 1mg, 3mg, 6mg, 9mg, and 12mg/g of the seeds. The germination studies were carried out according to the between paper method recommended by International Seed testing association [6].Dose range of fungicides was selected based on field prescribed concentration that could affect 10% to 95% of the seedlings with logarithmic intervals[7]. The seeds were allowed to germinate for 14days.Different parameters like Seed germination, Seedling length, Vigour Index, Chlorophyll content, Electrical conductivity and Phenolic content were

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recorded. Three sets in each concentration were maintained along with the control for comparison.

Percentage of germination

Seed germination was recorded in each experimental Set and it is calculated according to ISTA (1999).

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Seedling Length

The Root length and Shoot length of the germinated seeds were measured from each experimental set and expressed in centimeter.

Seedling Vigour Index

Seedling Vigour Index was computed by adopting the formula Suggested by Abdul-Baki and Anderson (08).
Seedling Vigour Index = Germination (%) x Seedling Length.

Electrical conductivity

The electrical conductivity was determined using Conductivity meter.

Chlorophyll

Chlorophyll content was estimated by Arnon method (09).

Total Phenol

Total Phenol was estimated by Malick and Singh (10).

Statistical analysis

The data obtained were subjected to analysis of variance using SPSS package version 20.0. The data are expressed as the mean analyzed by two way analysis of variance (ANOVA) and Scheffee was used as the test of significance.

RESULT AND DISCUSSION

Effect on Seed Germination

In Jaya cultivar seeds germination increases with increasing concentration of fungicide. Seed germination was maximum at 6 and 9mg concentration of fungicide.

Astik kumar found similar results in *Vigna radiata* treated with Bavistin. In Jyothi and IR variety maximum seed germination was observed at 3 and 6mg with 88.8 and 76.6% and at higher 9 and 12mg and lower 1mg seed germination was reduced (Table-1).

Root length

The effect of Carbendizium on root length was found statistically significant with treatment at various concentration of Carbendizium. In all three cultivars as compared to control, the root length was increased with increased concentration of fungicide. The maximum increased root length was observed in Jyothi cultivar at 3mg concentration (Table-2).

Shoot length

The data presented in the table-3 depicted in all three cultivars there was a significant increase in shoot length at all studied concentrations of carbendazium. However treatment at lower concentration (1mg, 3mg and 6mg), seedling length in all the 3 cultivars increases as compared to control. The maximum shoot length was found in Jyothi cultivar at 3mg concentration.

Electrical conductivity

Electrical conductivity (EC) is a method for measuring viability and seedling vigour. In Jaya, Jyothi and IR cultivars electrical conductivity was found to be increased as compared to control at all studied concentrations of fungicide as shown in table-4. In all the 3 cultivars Maximum increase in conductance was observed at 9 and 12 mg concentration.

Vigour index

As compared to control, the vigour index was found to be increased at all studied concentrations of fungicide. As shown in table-5the maximum vigor index was observed in Jyothi at 3mg concentration. Habtamu Ashagre in their experiment on seed germination and seedling growth of haricot bean cultivars showed phytotoxicity of shoot and root at higher concentration of copper sulphate. The shoot length, root length, and vigour index decreased significantly with increase in copper sulphateconcentration.

Chlorophyll a

In Jyothi and IR variety, as compared to control, Chlorophyll a content was found to be decreased at all the concentrations of

Table 1 Effect of Carbendazim on percentage of germination in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	75.33	76.40	75.23	78.80	77.63	82.43	77.63 ^a
	Jyothi	82.33	84.66	58.51	81.26	78.66	72.76	76.36 ^a
	IR	69.03	46.20	67.03	66.50	66.86	62.36	63.00 ^b
	Mean	75.56 ^a	69.08 ^a	66.92 ^a	75.52 ^a	74.38 ^a	72.52 ^a	72.33
	F value	Variety =11.083**					Concentration = 1.089**	
			Variety * Concentration = 1.884**					

Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.

the fungicides except at 9mg. Similar results found in wheat seedling treated with carbendazim reported by Rangwala tasneem. Pigment biosynthesis was found to be inhibited by the use of systemic fungicide.

In Jyothi and IR cultivar maximum decrease in Chlorophyll a content was observed at 3mg concentration which is considered to be the normal recommended dosage. In Jaya cultivar the Chlorophyll a content as compared to control was increased at higher dosage i.e., 9 & 12mg concentration(Table-6).

Table 2 Effect of Carbendazim on root length (cm) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	4.11	5.15	4.64	5.38	5.79	5.17	5.04 ^c
	Jyothi	5.96	10.56	10.22	7.83	8.94	9.32	8.80 ^a
	IR	6.66	5.83	8.03	8.77	7.75	7.97	7.50 ^b
	Mean	5.58 ^e	7.18 ^d	7.63 ^a	7.32 ^c	7.49 ^b	7.49 ^b	7.11
	F value	Variety =46774.687**			Concentration = 3774.345**			
		Variety * Concentration =2761.865 **						

Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.

Table 3 Effect of Carbendazim on shoot length (cm) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	3.96	4.05	3.63	4.63	4.64	3.97	4.14 ^c
	Jyothi	4.46	6.50	7.17	6.35	5.45	5.75	5.94 ^a
	IR	5.00	3.10	5.65	5.36	4.82	5.24	4.86 ^b
	Mean	4.47 ^c	4.55 ^c	5.48 ^a	5.45 ^a	4.97 ^b	4.98 ^b	4.98
	F value	Variety =2498.815**			Concentration =277.057 **			
		Variety * Concentration =339.893 **						

Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.

Table 4 Effect of Carbendazim on electrical conductivity (µS) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	539.66	627.00	659.33	797.00	681.00	654.66	659.77 ^a
	Jyothi	258.00	259.33	279.66	286.33	288.00	309.33	280.11 ^c
	IR	324.00	447.33	358.33	414.33	570.00	759.66	478.94 ^b
	Mean	373.88 ^c	444.55 ^{cd}	432.44 ^{de}	499.22 ^{bc}	513.00 ^{ab}	574.55 ^a	472.94
	F value	Variety =401.759 **			Concentration = 27.715**			
		Variety * Concentration = 17.244**						

Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.

Table 5 Effect of Carbendazim on vigour index in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	612	704	624	788	809	753	715 ^b
	Jyothi	863	1432	1456	1153	761	1098	1127 ^a
	IR	803	426	926	928	853	837	795 ^b
	Mean	759 ^a	854 ^a	1002 ^a	956 ^a	808 ^a	896 ^a	879
	F value	Variety =36.636**			Concentration = 3.186**			
		Variety * Concentration = 6.298**						

Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.

Table 6 Effect of Carbendazim on chlorophyll-a(mg/g fresh weight) content in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	0.351	0.330	0.126	0.241	0.482	0.667	0.366 ^a
	Jyothi	0.200	0.159	0.050	0.277	0.499	0.135	0.220 ^b
	IR	0.173	0.074	0.510	0.141	0.172	0.083	0.116 ^c
	Mean	0.241 ^c	0.188 ^c	0.076 ^f	0.220 ^d	0.384 ^a	0.295 ^b	0.234
	F value	Variety =3088.749**			Concentration =1048.879 **			
		Variety * Concentration = 495.887**						

Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.

Table 7 Effect of Carbendazim on chlorophyll-b (mg/g fresh weight) content in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	0.123	0.552	0.151	0.452	0.761	0.957	0.499 ^a
	Jyothi	0.451	0.293	0.068	0.312	0.774	0.224	0.354 ^b
	IR	0.262	0.167	0.270	0.232	0.249	0.185	0.228 ^c
	Mean	0.279 ^{cd}	0.337 ^{bc}	0.163 ^d	0.332 ^{bc}	0.595 ^a	0.455 ^b	0.360
	F value	Variety =48.528**			Concentration = 29.173**			
		Variety * Concentration = 22.440**						

Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.

Chlorophyll b

As compared to control Chlorophyll b content in Jaya cultivar was found to be Increased at all the studied concentrations of

parameters when treated with 9 and 12mg concentration. Hence a careful screening of dosage of fungicide should be done before the field application.

Table 8 Effect of Carbendazim on total chlorophyll (mg/g fresh weight) content in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	1.013	0.892	0.353	0.702	1.257	1.619	0.973 ^a
	Jyothi	0.653	0.447	0.122	0.591	0.562	0.372	0.458 ^b
	IR	0.427	0.246	0.122	0.367	0.432	0.272	0.311 ^c
	Mean	0.697 ^a	0.528 ^a	0.199 ^b	0.553 ^a	0.750 ^a	0.754 ^a	0.580
	F value	Variety =99.426**			Concentration = 18.248**			
		Variety * Concentration =7.660 **						

Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.

Table 9 Effect of Carbendazim on phenol content(μ g/fresh weight) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Carbendazim	Jaya	823.33	861.33	781.00	937.33	1016.33	949.66	894.83 ^a
	Jyothi	725.00	806.66	703.33	821.66	569.33	880.00	751.00 ^b
	IR	831.00	542.00	748.66	661.66	718.00	662.66	694.00 ^c
	Mean	793.11 ^{ab}	736.66 ^d	744.33 ^d	806.88 ^{ab}	767.88 ^{bc}	830.77 ^a	779.94
	F value	Variety =226.720**			Concentration = 14.329**			
		Variety * Concentration = 44.662**						

Means followed by the same letter within a column/row are not significantly different as indicated by Scheffe (P ≤ 0.05). **Significant at P ≤ 0.01.

the fungicides except at 3mg as shown in table-7. In Jyothi and IR cultivar Chlorophyll b content was found to be decreased at all studied concentrations of fungicide except at 9mg .

Total Chlorophyll

Total Chlorophyll content of Jaya, Jyothi and IR variety was found to be decreased at all studied concentrations of fungicide except at 9mg. As shown in table-8 in all the 3 cultivars Maximum increase in Total Chlorophyll content was observed at 9mg concentration. Increase in chlorophyll content by the use of systemic fungicide has also been reported in wheat (Wang & Waywood 1968), *Hibiscus esculentus* and *Capsicum annum* (Soaliha Ahmed & Siddiqui 1995). Klingersmith in 1961 reported that increases may be due to increase in the number of grana & intergrana space of chloroplast and also K⁺, Mg⁺, calcium ion up take during regulation of transpiration reported by Dyar in 1968. This affects photosynthesis & chlorophyll synthesis and thus results in increased chlorophyll content.

Phenolic contents

The phenolic content of rice cultivars treated with different concentration of Carbendazim are as shown in the table-9. In all the three cultivars as compared to control the phenolic content was increased with the treatment upto 9mg and there after it declined gradually at 12mg concentration. Similar results were found in white yam treated with triazole fungicide reported by Cheruth Abdul Jallel (2008).

CONCLUSION

The data obtained indicates that seed treatment with Carbendazim upto 6mg is recommended or applicable. However adverse effects were observed in all growth

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