

Impact of Mancozeb Stress on Seedling growth, Seed Germination, Chlorophyll and Phenolic Contents of Rice Cultivars

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Abstract: *The present work was carried out to study the effect of mancozeb on seed germination, seedling length, vigor Index, Chlorophyll a, Chlorophyll b, Total Chlorophyll, electrical conductivity, conductance and phenolic contents. The experiments were carried out for 14 days after soaking the seeds in different concentration of Mancozeb and a control was maintained. A two way analysis of variance showed that the difference in the rate of seed germination and other parameters was statistically significant among different concentration of treatment of fungicide. It was found that concentration of 1, 3, 6 mg has stimulatory effect on seed germination, root length, shoot length, vigour index, chlorophyll and phenolic contents and concentration of 9 and 12mg showed inhibitory effect on germination and other parameters.*

Keywords: Mancozeb, seed germination, electrical conductivity, Total Chlorophyll, and Phenolic contents.

1. Introduction

Seed dressing with fungicide is a conventional method used for the control of seed & soil borne pathogen that results in healthy plant development and increases the crop yield(1). Although the seed treatments protect the seed & seedlings from pests & disease, it causes secondary effects on germination & growth (2). Mancozeb, organic contact fungicide have preventive action by killing or inhibiting fungi or spores before the mycelia grow and develop within the plant tissues (3). They undergo transformation to ethylene di isothiocyanate, which inactivates thiol groups of enzymes and metabolites in fungal cells. These compounds control broad range of fungi at relatively low application rates (4). Therefore the present study was undertaken to know the phytotoxicity of mancozeb fungicide on percentage of seed germination, vigor index, chlorophyll a, chlorophyll b, Total chlorophyll, conductance, phenolic contents of rice cultivars.

2. Literature Survey / Related Work

Bader & Abdel Basset (1999) showed that triforin type fungicides strongly inhibit electron transport reactions of chloroplast. Fungicides become phytotoxic retarding plant growth and reducing photosynthesis (5). Repeated use of fungicides produces chlorosis, irregular depression at the central & marginal portions of leaf sharp decrease in cell division and also inhibit the seeding growth(6). Phenol was selected as stress indicator as it causes stress and this contribute to increased total phenolic content in plant (7).

3. 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 10 minutes 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 mancozeb (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 (8). Dose range of fungicides was selected based on field prescribed concentration that could affect 10% to 95% of the seedlings with logarithmic intervals (9). The seeds were allowed to germinate for 14days. Different parameters like Seed germination; Seedling length, Vigour Index, Electrical conductivity, Chlorophyll content, and Phenolic content were studied. Three sets in each concentration were maintained along with the control for comparison.

3.1. Percentage of Germination

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

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

3.2. Seedling Length

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

3.3. Seedling Vigour Index

Seedling Vigour Index was computed by adopting the formula Suggested by Abdul-Baki and Anderson (10).
Seedling Vigour Index = Germination (%) x Seedling Length.

3.4. Electrical Conductivity

The electrical conductivity was determined using Conductivity meter.

3.5. Chlorophyll

Chlorophyll content was estimated by Arnon method (11).

3.6. Total Phenol

Total Phenol was estimated by Malick and Singh (12).

3.7. 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.

4. Results and Discussion

4.1. Effect on Seed Germination

The Percentage of seed germination in control and fungicide treated seedling is presented in table 1. The rates of seed germination in Jaya cultivar increases progressively with increasing concentration of fungicide. In Jyothi and IR cultivar, the percentage of germination was decreased with higher concentration of fungicides, (6, 9, 12mg of Mancozeb). The increases in Germination % might be due to the decrease of seed microflora (13). Sammaiah have showed pesticide induced alterations in physiological response in *Solanum melangina* (L).

4.2. Root Length

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

4.3. Shoot Length

The data presented in the table-3 depicted significant decline in shoot length with treatment of mancozeb at the higher concentration. However treatment at lower cone in 1mg, 3mg, and 6mg seedling length in all the 3 variety increases as compared to control. The maximum shoot length was found in Jyothi variety at 3mg concentration.

4.4. Vigor Index

In all three cultivars, as compared to control the vigor index was increased with treatment up to 6mg and there after it declined gradually towards higher concentration 9mg and 12mg. The difference in vigor index was also found significant with treatment at different concentration. The maximum vigor index was observed in Jyothi variety at 3mg concentration (Table-4). Habtamu Ashagre (2013) in their experiment on seed germination and seedling growth of haricot bean cultivars showed phytotoxicity of shoot and root length at higher concentration of copper sulphate. The shoot length, root length and vigor index decreased significantly with increase in copper sulphate concentration.

4.5. Electrical Conductivity

The electrical conductivity of rice cultivars treated with different concentration of mancozeb is as shown in the table-5. In all the varieties as compared to control conductance was found to be increased at all studied concentrations of fungicide. In all the 3 varieties Jaya, Jyothi and IR variety maximum increase in conductance was observed at 9mg and 12mg concentration. Higher electrical conductivity of seed leachate indicates lower germination potential.

4.6. Chlorophyll a

As compared to control Chlorophyll a content of Jaya cultivar was found to be increased at 1mg and 3mg and decreased at 6mg, 9mg and 12mg concentration as shown in table-6. In Jyothi cultivar Chlorophyll a content was found to decreased at all studied concentrations of fungicide as compared to control but at 6mg concentration it was found to similar to control. In IR cultivar as compared to control Chlorophyll a content was found to be increased at all studied concentrations of fungicide. Maximum increase in Chlorophyll a content was observed at 1mg concentration (Table-6). This result was in accordance with Jagatheeswari where the influence of mercuric chloride on seed germination, seedling growth and biochemical analysis of green gram were studied.

4.7. Chlorophyll b

Chlorophyll b content of Jaya and Jyothi cultivar was found to be decreased at all studied concentrations of fungicide as compared to Control as shown in table-7. But in IR cultivar there is significant increase Chlorophyll b content up to 9mg and there after it decreases at 12mg concentration.

4.8. Total Chlorophyll

As shown in table-8 Total Chlorophyll content of Jaya, Jyothi cultivar decreased with increased concentrations of fungicide as compared to control. But in IR cultivar Total Chlorophyll content increase up to 9mg and at 12mg concentration it decreased.

4.9. Phenolic Contents

The phenolic content of rice cultivars treated with different concentration of mancozeb is as shown in the table-9. In all the three varieties as compared to control the phenolic content was increased with the treatment upto 9mg and there after it declined gradually at 12mg concentration. Soaliha Ahmed and Zamin and Shaheed Siddiqe in their experiment on the effect of Topsin fungicide on chlorophyll, protein and phenol contents (*Hibiscus esculents* and *Capsicum annum*) also found similar results. Phytotoxic in the form of polyphenols effect biochemical pathways. Plants fungicide treatments are under chemical stress and this may act as a protective compound against pathogenic fungi (11)

5. Conclusion

The data obtained indicates that germination percentage, electrical conductivity vigour Index, Chlorophyll a,

Chlorophyll b, total Chlorophyll and phenolic contents were slightly affected at 9 and 12mg concentration. Treatment at 1,3,6mg concentration shows better stimulating effect on seed germination and plant growth as compared to control. Therefore the usage of fungicide beyond threshold concentration should be avoided for the better crop yield.

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Table 1: Effect of Mancozeb 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	
Mancozeb	Jaya	75.00	77.66	78.33	83.00	85.00	82.33	80.22 ^b
	Jyothi	81.33	80.66	88.66	88.73	85.00	82.33	84.45 ^a
	IR	68.56	65.73	72.43	76.60	68.43	68.00	69.96 ^c
	Mean	74.96 ^c	74.68 ^c	79.81 ^b	82.77 ^a	79.47 ^b	77.55 ^b	78.21
	F value	Variety =535.343**			Concentration =46.625 **			
Variety * Concentration =10.978 **								

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

Table 2: Effect of Mancozeb 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	
Mancozeb	Jaya	8.66	9.97	10.66	11.18	10.25	11.22	10.32 ^b
	Jyothi	8.86	11.23	12.55	11.34	12.14	12.22	11.39 ^a
	IR	6.73	6.74	8.07	7.58	7.91	7.26	7.38 ^c
	Mean	8.08 ^c	9.32 ^d	10.43 ^a	10.03 ^c	10.10 ^{bc}	10.23 ^b	9.70
	F value	Variety =8122.811**			Concentration =725.817 **			
Variety * Concentration = 114.793**								

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

Table 3: Effect of Mancozeb 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	
Mancozeb	Jaya	6.26	7.56	7.92	8.38	6.25	7.16	7.26 ^b
	Jyothi	6.67	7.25	8.56	8.30	8.55	8.18	7.92 ^a
	IR	4.00	4.92	4.92	5.95	4.74	4.93	4.91 ^c
	Mean	5.64 ^d	6.58 ^c	7.13 ^{ab}	7.54 ^a	6.51 ^c	6.76 ^{bc}	6.69
	F value	Variety =553.929**		Concentration =45.751 **				
	Variety * Concentration =9.489 **							

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

Table 4: Effect of Mancozeb 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	
Mancozeb	Jaya	1092	1332	1506	1605	1396	1470	1400 ^b
	Jyothi	1308	1481	1877	1722	1739	1633	1627 ^a
	IR	806	765	976	1036	870	826	875 ^c
	Mean	1096 ^t	1193 ^c	1443 ^b	1454 ^a	1335 ^c	1310 ^d	1301
	F value	Variety =177573.261**		Concentration = 13181.243**				
	Variety * Concentration = 1653.413**							

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

Table 5: Effect of Mancozeb 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	
Mancozeb	Jaya	438.00	560.00	635.00	709.33	625.66	737.66	617.61
	Jyothi	431.66	428.66	437.00	453.00	510.00	598.66	476.50
	IR	334.00	429.33	396.00	409.00	441.00	452.00	410.22
	Mean	401.22	472.66	489.33	523.77	525.55	596.11	501.44
	F value	Variety = 79.837**		Concentration = 14.987**				
	Variety * Concentration =3.216 **							

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

Table 6: Effect of Mancozeb 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	
Mancozeb	Jaya	0.263	0.312	0.313	0.144	0.073	0.051	0.192
	Jyothi	0.091	0.022	0.073	0.091	0.026	0.028	0.554
	IR	0.022	0.144	0.102	0.084	0.087	0.036	0.079
	Mean	0.126	0.159	0.163	0.106	0.062	0.038	0.109
	F value	Variety =405.644**		Concentration = 97.430**				
	Variety * Concentration =59.465**							

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

Table 7: Effect of Mancozeb 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	
Mancozeb	Jaya	0.406	0.314	0.314	0.253	0.051	0.368	0.284 ^a
	Jyothi	0.136	0.074	0.053	0.140	0.072	0.018	0.082 ^b
	IR	0.046	0.150	0.074	0.081	0.091	0.072	0.085 ^b
	Mean	0.196 ^a	0.179 ^a	0.147 ^a	0.158 ^a	0.071 ^a	0.152 ^a	0.151
	F value	Variety =18.718**		Concentration =1.287 **				
	Variety * Concentration = 1.599**							

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

Table 8: Effect of Mancozeb 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	
Mancozeb	Jaya	0.694	0.534	0.650	0.420	0.136	0.246	0.447
	Jyothi	0.240	0.093	0.127	0.203	0.093	0.043	0.133
	IR	0.072	0.291	0.121	0.160	0.255	0.114	0.169
	Mean	0.335	0.306	0.299	0.261	0.162	0.134	0.249
	F value	Variety =159.993**			Concentration = 18.811**			
	Variety * Concentration =18.993 **							

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

Table 9: Effect of Mancozeb on phenol content (µg/fresh weight) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentration of Fungicide (mg /gm)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	793.66	975.33	1521.33	2027.00	1722.33	1898.00	1489.61a
	Jyothi	557.66	456.33	380.00	636.66	810.00	652.33	582.16b
	IR	645.33	407.33	548.00	566.00	718.33	569.33	575.72b
	Mean	1002.22	613.00	816.44	1076.55	1083.55	916.44	918.03
	F value	Variety =2005.442**			Concentration = 182.756**			
	Variety * Concentration =85.237**							

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