Mancozeb Induced Changes in Carbohydrate Metabolism of Paddy Cultivars during Seed Germination

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Abstract: ***Fungicides may cause oxidative stress in plants. Plants adapt to stress by different mechanism including changes in physiological and biological processes. Carbohydrates are considered as important metabolites in plants under stress condition. The reduction in their synthesis may affect plant growth and yield. The present work is an attempt to understand the effect of different concentrations of mancozeb on carbohydrate metabolism in paddy cultivars. Seed treatment with mancozeb influenced different aspects both at morphological and biochemical level. The study was carried out for 14 days after soaking the seeds in different concentrations of mancozeb and control was maintained. There was considerable significant positive increase in starch content, reducing sugar, β-amylase and invertase enzyme activities in Jyothi cultivar. Thus mancozeb acts as stimulatory effector. This may be adoptive measure under stress condition. Where as in Jaya and IR-64 cultivar, mancozeb treatment was found to be having detrimental effect.***

Keywords: Mancozeb, total carbohydrate, starch, reducing sugar, α and β amylase and invertase

1. Introduction

Rice is the most economically important staple food crop for more than half of the world's population. To meet the increasing demand of the rice supplies, usage of the improved cultivars and the intensive cultural practices have been done which resulted in the occurrence and severity of disease infesting rice in several countries [1]. The major rice diseases that cause economic losses are rice blast, sheath blight, Bacterial blight and Tungro virus disease [2]. The integrated disease management includes growing resistant varieties, biological and chemical methods. Pre-sowing seed treatment with fungicide is an efficient and economic means of plant protection for pests [**3**]. However application of the fungicide on crop plant can increase or decrease the quantative formation of various biomolecules and thereby alter the physiological parameters of growth [4]. The degree of damage varies with the plant species, their active ingredients, dosage and frequency of the pesticide application [5].

The fungicide treatment may have positive and negative effect on the metabolism during seed germination which can be analyzed by evaluating metabolic processes in treated seeds. Therefore the present study was aimed to assess the effect of different concentrations of mancozeb on seed reserves carbohydrates contents like reducing sugar, total carbohydrate, starch and the effect on activity of the enzymes like α-amylase, β-amylase and invertase in germinating seed of rice cultivars-Jaya, Jyothi and IR-64.

2. Materials and Methods

2.1 Collection of seed samples and treatment

Paddy seeds (Jaya, Jyothi, and IR-64 cultivars) 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 [6]. The seeds were allowed to germinate for 14 days and then processed for further studies. Three sets in each concentration were maintained along with the control for comparison.

2.2 Extraction and estimation of Starch and Total carbohydrate

Extraction of starch and total carbohydrate was carried out as described by Hedge and Hofreiter [7] and it was estimated by Anthrone method [8].

2.3 Extraction and estimation of reducing sugars

Sugars were extracted twice with 80% ethanol at 90^{o}C followed by 4 times extraction with 70% ethanol as described by Prabhjot Kaur Gill [9]. From this extract the reducing sugars were quantitatively estimated by DNS method [10].

2.4 Extraction of α-amylase, β-amylase and Invertase and determination of their activity

The extraction of α-amylase and β-amylase was carried out by following Gupta *et al.*, [11]. Invertase activity was assayed by Mahadevan and Sridhar [12].

2.5 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.

3. Results and Discussion

3.1 Effect of mancozeb on total carbohydrates:

Carbohydrate serves as vital source of energy in plants and provides carbon skeleton for the synthesis of organic compounds. In addition to this, it also functions as signaling molecule [13]. The results of the present study shows that as compared to control, the total carbohydrate in Jaya cultivar was found to be increased at 3, 9 and 12mg while 1 & 6mg concentration showed a decline in total carbohydrate (Table 1). The total carbohydrate content was found to be maximum at 3 mg concentration with 632 mg/g fresh weight. Plants in response to environmental stress accumulate sugars in different parts of the plants [14]. Adaptation to these stresses has been attributed to the stress induced increase in carbohydrate levels. Similar to our results, Saladin *et al.*, (2003)[15] have reported higher concentration of flumioxazin induces an accumulation of soluble sugar in *Vitis Vinifera*.L. Hennouni *et al.*, (2012) [16] also observed increased total carbohydrate in wheat varieties treated with combination of cyproconazole and propiconazole. Bordjiba *et al.*, (2009) [17] have showed higher rates of carbohydrates in wheat varieties treated with hexaconazole. Further fungicide molecules interact with electron transport system [18] and increases NAD, NADP ratio and ATP and thus increase the total carbohydrates[19]. On the contrary in Jyothi cultivar and IR-64 the mancozeb treatment was found be having inhibitory effect at all studied concentrations and showed reduced amount of total carbohydrates. Reduction of carbohydrate contents may be due to reduced synthesis, damage of vascular tissues or it may be due to the influence of fungicides on enzymes involved in carbohydrate metabolism. Zamin Siddiqui and Ahmed (2002) [20] suggested that the toxicants produced from the fungicides alters cytochrome oxidase activity and block the respiratory pathway. The results were similar to the effect of triazophos in spinach (Kengar *et al.*, 2014) [21]. Similarly Manzoor Ashrafi and Goutam Pandit (2015)[22] also reported decrease in total carbohydrate content in brinjal and cabbage treated with carbofuran and endosulfan. Rajabi *et al.*,(2012) [23]have also showed a significant reduction in total carbohydrate in wheat seedlings treated with metribuzin.

3.2 Effect of mancozeb on starch

In the present study the increased magnitude of starch content over the control was observed at 3 and 6 mg concentrations in Jaya cultivar and the value decreased significantly at 1, 9 and 12 mg concentrations (Table 2). In Jyothi cultivar the starch content was found to be increased over the control at all treatments and 9 mg concentration showed maximum starch content with 207 mg/g. Thus it has a stimulatory effect on starch content. Treatment of IR-64 cultivar with 1 and 6mg showed positive increase in starch content over the control and in other treatments starch content was significantly reduced. Accumulation of starch due to stress may impair photo assimilation and uptake of sugar (24). Kamble and Samble (1999) [25] have also found that starch content in trigonella seedlings increased with increased concentrations of carbendazim. Similarly Kenger *et al.*, (2014) [21] reported that hexaconazole increases starch content in the germinating seeds of spinach and gaur. Moumit Roy *et al.*, (2013) [26] also reported increased insoluble sugar content in egg plant treated with carbaryl insecticide.

3.3 Effect of mancozeb on reducing sugar

As compared to untreated seedlings, reducing sugar was found to be reduced at all treated concentrations in Jaya cultivar. However treatment with 1mg showed increased sugar content with 5.13 mg/g. In IR-64 cultivar the reducing sugar was found to be reduced at all treatments except at 9mg concentration which showed almost similar effect as control. Reduction of reducing sugar may be due to inactivation of RUBP carboxylase activitiy (27) or low degradation of starch (28) and due to stress exerted by the herbicides (29).

While in Jyothi cultivar the effect was reversed. The reducing content was increased at 1, 3, 9 &12mg concentrations. 9mg concentration showed increased reducing sugar with 2.32 mg/g while 6mg (recommended dosage) showed the minimum reducing sugar with 0.48 mg/g (Table 3). This may be due to enzymatic changes which are responsible for the conversion of starch to reducing sugar (30) or may be due to non conversion to non reducing sugar (31). Plants in response to environmental stress accumulates sugars in different parts and accumulation of these sugars help in the development of desiccation tolerance (32) and vitrification and thus avoids the damage to cells caused by crystallization (33). Hence this might be the mechanism adapted to reduce the fungicidal stress.

3.4 Effect of mancozeb on α-amylase activity

A strong inhibition of α-amylase activity was observed in Jaya and Jyothi cultivars. Thus mancozeb treatment showed detrimental effect at all concentrations and resulted in reduced activity (Table-4). This inhibitory effect may be due to a failure in the reserve mobilization process from cotyledons. Mann *et al.*, (1965) [34] also reported that inhibition of protein synthesis may result in the decreased amylase production. Inhibitors of α-amylase are present in the seed of many cereals and in storage vacuoles of cotyledonary cells and thus exhibit inhibitory effects on α-amylase [35]. Prasad and Mathur (1983) [36] reported the reduced α-amylase activity in germinating seeds of *Vigna mungo* treated with varying concentrations of metasystox and cuman.

Gibberillic acid (GA) is known to induce the synthesis of α-amylase [37]. Mamdouh *et al.*, (1998) [38] suggested that the loss of endogenous gibberillic acid decreases α-amylase activity and also they showed that metolachlor provoked a significant loss of α-amylase activity in *Zea mays* during germination and growth processes. Wilkinson (1988) [39] showed that alachlor and metolachlor inhibited gibberillic acid synthesis in sorghum seedlings and thus induces a decrease in amylase synthesis. In IR-64 cultivar there was no much difference between the control and 1 mg concentration. 6mg, 9mg and 12 mg concentrations showed slight increased activity over the control while the activity was slightly declined at 3 mg concentration (Table 4). Further the results of the present study are in conformity with the observations of Anwarul Islam *et al.,* (2003) [40], Shrikant Jain (2012) [41] and Chibi Fatiha (2011) [42]. Treatment of pesticides may cause an osmotic stress and thus causes alternation in membrane structure. These molecules can interact with pesticide and thus it affects the production of enzyme protein [43] and under stress condition lysosomes are broken down resulting in increased level of hydrolytic enzymes [44].

3.5 Effect of mancozeb on β-amylase activity:

In Jaya cultivar the activity of β-amylase was found to be increased at 3, 6, and 12mg concentrations & decreased at 1 and 9mg concentrations as compared to control. While in Jyothi cultivar the activity of β-amylase was increased at 12mg concentration but decreased at other treatments. In IR-64 the effect of mancozeb was detrimental and the activity of β-amylase was found to be decreased over the control at all studied concentrations (Table 5). Jha and Dubey (2005) [45] reported a decline in the activity of β-amylase in endosperm as well as embryoaxis of germinating rice seedlings exposed to arsenic toxicity. Veer Pratap and Sharma (2010) [46] and Evan Jebopry (2012) [47] reported decreased β-amylase activity with increasing water stress in seedlings of *Pisum sativ*um and *Phaseolus mungo.*

The Anova for the mean values of the activity of β-amylase activity showed that IR-64 cultivar was found to be highly significant compared to Jaya and Jyothi cultivar.

3.6 Effect of mancozeb on invertase activity:

As compared to control, invertase activity in Jaya cultivar treated with mancozeb was found to be enhanced at 1, 6, 9, and 12mg concentrations and at 3mg concentration invertase activity was found to be similar to control. In IR-64 the activity of invertase was found to decreased over the control at all treatments. Increase trend was observed in Jyothi cultivar up to 9mg concentration while at 12 mg concentration it showed slightly reduced activity as compared to control (Table 6).Two fold increase in the invertase activity was reported in *Sorghum bicolor* exposed to salt stress and ABA by Meenu Thakur and Arun Dev Sharma (2005) [48], further they suggested that the induction of invertase was an important component of stress response. Invertases have a regulatory control in the carbohydrate mobilization and also during environmental stress [49]. Inhibitory effect of mancozeb treatment in IR -64 cultivar may be due to reduced conversion of total sugars to hexoses which in turn reduced the synthesis of sugar nucleotides required for the cellular synthesis and growth [50]. Hawker and walker (1978) [51] stated that reduced invertase activity may be due to decreased metabolic activities.

4. Conclusion

In the present investigation seed treatment with different concentrations of mancozeb induced changes in carbohydrate content and the enzymes of the carbohydrate metabolism has been reported. This change might be involved for acclimation of germinating seeds. Further investigation is required to understand the regulatory pathway and molecular mechanism involved in seed acclimation.

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Table 1: Effect of mancozeb on total carbohydrates (mg g^{-1} fresh wt) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	268.0	112.0	632.0	100.0	460.0	504.0	346.0^{a}
	Jyothi	248.0	64.0	184.0	212.0	90.0	146.0	157.3^{c}
	IR-64	240.0	150.0	105.0	155.0	200.0	130.0	163.0^{b}
	Mean	252.0^{c}	108.6^{f}	307.0^{a}	155.6^{e}	250.0^{d}	260.0^{b}	222.0
	F value	Variety =206994.6**			Concentration = 49675.0**			
		Variety * Concentration = 58679.0**						

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 starch content (mg g^{-1} fresh wt) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	447.3	271.8	524.7	460.4	238.2	208.0	358.4^{a}
	Jyothi	79.2	205.2	136.8	97.2	207.0	132.4	142.9^{b}
	IR-64	114.0	195.3	72.0	190.2	81.0	71.1	120.6^{c}
	Mean	213.5^{d}	224.1^{c}	244.5^{b}	249.2^{a}	175.4^{e}	137.1^{f}	207.3
	F value	Variety = 322388.68**			Concentration =175643887**			
		Variety * Concentration = 29107.63**						

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 reducing sugar (mg g^{-1} fresh wt) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	5.20	5.13	4.60	4.40	3.80	3.80	4.48^{a}
	Jyothi	0.72	0.96	0.80	0.48	2.32	0.80	1.01^{b}
	IR-64	0.59	0.26	0.53	0.33	0.59	0.53	0.47^{c}
	Mean	2.17^{ab}	2.11^{b}	1.97^{c}	1.73^{d}	2.23^{a}	1.71^{d}	1.99
	F value	Variety = 28758.56**			Concentration = 154.166**			
		Variety * Concentration = 343.456**						

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 α-amylase activity (μmoles of maltose released $min^{-1}g^{-1}$) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	34.46	33.63	29.30	34.47	33.33	27.91	32.18^{b}
	Jyothi	35.35	29.11	29.90	31.99	30.62	25.64	30.43^{b}
	IR-64	70.11	70.33	67.43	71.35	79.37	71.77	71.73^{a}
	Mean	46.64^{a}	44.36^{a}	42.21^{a}	45.94^{a}	47.77^{a}	41.77^{a}	44.78
	F value	Variety = 549.796**			Concentration = 2.985**			
		Variety * Concentration = 1.425**						

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 β- amylase activity (µmoles of maltose released $min^{-1}g^{-1}$) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	30.70	27.55	33.81	36.20	29.20	32.40	31.64[b]
	Jyothi	23.13	13.96	17.40	8.56	21.51	32.55	19.52[c]
	IR-64	82.70	76.00	75.70	77.46	76.16	79.60	77.93[a]
	Mean	45.51[ab]	39.17[c]	42.30[bc]	40.74[c]	42.29[bc]	48.18[a]	43.03
	F value	Variety = 4110.099**			Concentration = 23.311**			
		Variety * Concentration = 19.298**						

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 mancozeb on invertase activity (µmoles of glucose released $min^{-1}g^{-1}$) in germinating seeds of rice cultivars

Fungicide	Rice cultivars	Control	Different concentrations of fungicide (mg/g)					Mean
			1	3	6	9	12	
Mancozeb	Jaya	7.02	9.84	7.05	14.89	13.48	9.99	10.38[c]
	Jyothi	15.40	17.90	20.30	16.49	19.89	15.26	17.54[b]
	IR-64	73.56	71.20	57.06	64.13	65.70	71.37	67.17[a]
	Mean	31.99[a]	32.98[a]	28.14[b]	31.83[a]	33.02[a]	32.20[a]	31.69
	F value	Variety = 6553.626**			Concentration = 11.255**			
		Variety * Concentration = 24.645**						

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.