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Original Research Paper

Effect of Tricyclazole on the Growth and Metabolism of *Zea mays*

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ABSTRACT

Hybrid maize (MHM 9001) seeds were treated with varied concentrations of Tricyclazole, a systemic fungicide used to control blast disease of rice. The main composition of Tricyclazole is 70% sodium lauryl sulphate. It is used for both seed treatment and foliar spray. Morphological and biochemical parameters were analysed. Multiple sets were prepared for comparison with untreated sets. All parameters were analysed using standard methods and the results were subjected to 2 tailed " t" test and Pearson's Correlation Matrix and also Bray-Curtis similarity index. The tables were analysed to know the effect of Tricyclazole on cultivar of maize. A strong positive correlation exists between Vigour index and DNA purity in the samples treated with the fungicides, and a negative correlation between root length and fresh weight of the samples. 94% similarity was seen between chlorophyll-*a* and chlorophyll-*b*; and shoot length and fresh weight when Bray-Curtis similarity index was applied.

INTRODUCTION

Very little information is available on the effect of fungicides and other pesticides on pulses and cereals, in general, and maize in particular. Some of the important studies on the effects of fungicides on various plants include those of Garcia (2004), Mahfouz (2011) and Reddy & Vidyavathi (1983).

Triazoles are a group of fungicides and plant-growth retarding chemicals, which contain three conserved nitrogen atoms within a five-member ring. Several of these compounds, including paclobutrazol, uniconazole, tetraconazole and triadimefon, cause remarkable growth responses in plants. Changes caused by triazoles are mediated through cytochrome P-450 group of enzyme inhibition (Sopher et al. 1999, Taiz & Zeiger 1998, Zhu et al. 2004). There is evidence that triazoles applied to plants could be a means to alleviate water stress. In fact, plant responses such as increased ABA, proline, antioxidants and stomatal closure were observed after triazole treatment which appear to mimic some drought avoidance or tolerance mechanisms. This has been reported in wheat (Berova & Zlatev 2003, Gilley & Fletcher 1997), tomato (*Lycopersicon esculentum*) (Still & Pill 2004), perennial rye grass (*Lolium perenne*) (Jiang & Fry 1998), and silver maple (*Acer saccharinum*) (Marshall et al. 2000).

Maize is extensively cultivated in Mysore district where the excess use of insecticides and pesticides has reduced its yield considerably. With a view to understand the effect of fungicide Tricyclazole on the growth and seedling vigour and variation in morphological and biochemical characteristics of maize, the present work was undertaken.

MATERIALS AND METHODS

The seeds of hybrid *Zea mays* (MHM 9001) were of Mudra Seeds Pvt Limited, Hyderabad sold locally. The seeds were treated with thiram to store them for longer time. They were soaked in fungicide at concentrations of 0.1%, 0.2% and 0.3% for 24 hours. Tricyclazole (75% W.P), recommended for the leaf blast of paddy, was purchased from Bharath Insecticides Pvt Ltd, Mumbai. The seeds were allowed to germinate by paper method for 15 days (Nene & Thapliyal 1993). The seeds were then analysed for morphological and biochemical parameters. Control was maintained by soaking the seeds in water for 24 hours. Three sets for each treatment were maintained.

Seedling vigour index (SVI) was calculated by the modified formula of Abdul-Baki & Anderson (1973); conductivity test was used to quantify the leakage of electrolytes from the seed coat (Hendricks & Taylorson 1976); and chlorophyll was extracted in 80% acetone and determined by Arnon method (Arnon 1949). Reducing sugars were estimated by dinitro salicylic acid method (Miller 1959), total carbohydrates and starch by the anthrone method (Hedge 1962), and total proteins by Lowry's method (Lowry et al. 1951). Isolation of DNA was done by chloroform and isoamyl alcohol mixture in 24:1 ratio (Murmur 1961) and purity of DNA was determined by the method of Hillary Luebbehusen (2004). DNA was estimated by DPA method (Burton 1956), and RNA by orcinol method (Bial 1902). The data obtained were subjected to statistical analysis for conclusive determination.

Table 1: Effect of Tricyclazole on the metabolism of Zea mays.

Sl No	Parameters	Control	0.1%	0.2%	0.3%
Morphological parameters					
01	Vigor Index	2701.0 ± 1	474.2 ± 0.2***	1084.1 ± 0.1***	788.7 ± 0.1***
02	Shoot Length (cm)	5.2 ± 0.2	9.27 ± 1.50*	10.02 ± 0.01***	7.48 ± 0.07**
03	Root Length (cm)	18.2 ± 0.1	13.5 ± 0.1***	8.18 ± 0.20***	18.82 ± 0.16*
04	% Germination	90.00 ± 10	20.00 ± 10**	60.00 ± 10	30.00 ± 10**
05	Fresh Weight (g)	6.8 ± 0.1	8.5 ± 0.1*	10.4 ± 0.2***	6.2 ± 0.1***
06	Seed Weight (g)	3.33 ± 0.31	5.53 ± 0.25*	5.200 ± 0.1**	4.200 ± 0.173
07	Leaf weight (g)	1.17 ± 0.25	0.73 ± 0.35	0.46 ± 0.17*	0.493 ± 0.090*
08	Root Weight (g)	2.25 ± 0.25	2.30 ± 0.10	4.8 ± 0.1**	1.3 ± 0.1*
Biochemical Parameters					
09	Conductivity (μmhos)	352.00 ± 5.29	246.33 ± 4.04***	285.67 ± 6.03**	82.33 ± 8.74
10	Chlorophyll *a* (μg/mL)	0.015 ± 0.001	0.023 ± 0.001*	0.008 ± 0.002*	0.030 ± 0.002*
11	Chlorophyll *b* (μg/mL)	0.008 ± 0.001	0.022 ± 0.001*	0.007 ± 0.002	0.030 ± 0.017*
12	Total chlorophyll (μg/mL)	0.025 ± 0.001	0.045 ± 0.001***	0.015 ± 0.001*	0.046 ± 0.015
13	Reducing sugars (mg/g Fwt)	200 ± 100	233.3 ± 152.8	300 ± 100	200 ± 100
14	Total carbohydrates (mg/g Fwt)	300 ± 100	283.3 ± 125.8	500 ± 200	723.3 ± 25.17*
15	Non reducing sugars (mg/g Fwt)	900 ± 100	906.6 ± 100.67	643.33 ± 51.32*	1070 ± 62.5*
16	Total proteins (mg/g)	0.43 ± 0.2	0.10 ± 0.05	0.24 ± 0.14	0.13 ± 0.1*
17	DNA quantification	0.13 ± 0.03	0.023 ± 0.005*	0.10 ± 0.05	0.03 ± 0.01
18	DNA content	0.64 ± 0.15	0.5 ± 0.1*	0.53 ± 0.25	0.53 ± 0.21
19	DNA	120 ± 20	60 ± 20*	30 ± 10*	50 ± 10*
20	RNA	190 ± 10	156.7 ± 15.3	130 ± 30.0	120 ± 21.8*

***Values extremely significant. Mean values followed by SE at 0.05 significance level
**Values very significant, *Values significant

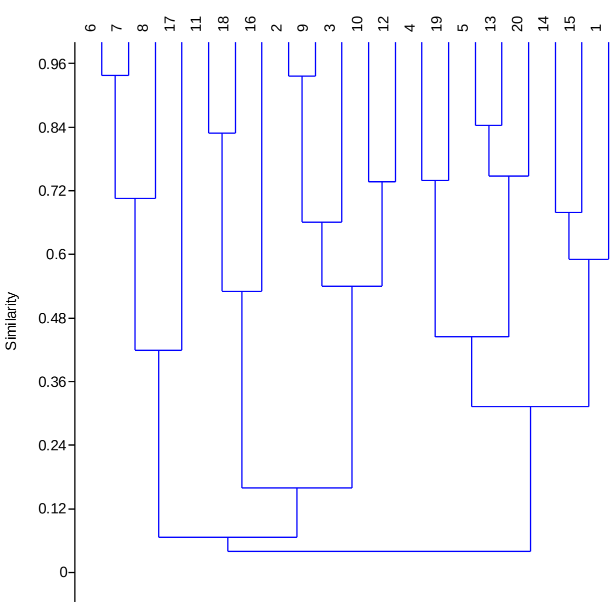

Fig. 1: Bray-Curtis similarity index.
(1. Vigour index, 2. Shoot length, 3. Root length, 4. % Germination, 5. Conductivity, 6. Chlorophyll-*a*, 7. Chlorophyll-*b*, 8.Total chlorophyll, 9. Fresh weight, 10. Seed weight, 11. Leaf weight, 12. Root weight, 13. Reducing sugar, 14. Non reducing sugar, 15. Total carbohydrates, 16. Proteins, 17. DNA quantification, 18. DNA content, 19. DNA estimation, 20. RNA estimation)

RESULTS

The results of the treatments along with a paired 't' test are presented in Table 1 for the morphological and biochemical parameters. Significantly higher vigour index was obtained in all the concentrations of the fungicide treatment. Shoot length was also significantly more in all the treatments. Root length was significantly reduced in 0.1% and 0.2% concentrations, but increased in 0.3% treatment. Chlorophyll was reduced in 0.2% treatment, but increased in other treatments. Reducing sugar was lower in 0.2 % treatment, while total proteins were lower in all the treatments. DNA and RNA contents were also lower in all the treatments as compared to control.

In order to understand the relation between all the parameters, the data in Table 1 were subjected to Pearson's correlation matrix (Table 2). As per the Pearson's correlation matrix, a very high significance at 0.01% level was observed between root length and fresh weight, which are inversely proportional, while vigour index and DNA content were positively correlated. Vigour index was correlated to proteins; shoot length to seed weight; percent germination to proteins and DNA; fresh weight to reducing sugar; leaf weight to DNA and RNA, root weight to reducing sugar; and total carbohydrates and proteins to DNA. All these are positively correlated clusters, while root length is negatively

Table 2: Pearson's correlation matrix for morphological and biochemical parameters.

~	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
2	-0.812	1																		
3	0.355	-0.84	1																	
4	0.945	-0.6	0.062	1																
5	0.652	-0.183	-0.329	0.725	1															
6	-0.428	-0.18	0.692	-0.673	-0.808	1														
7	-0.64	0.07	0.489	-0.82	-0.904	0.964	1													
8	-0.526	-0.045	0.566	-0.772	-0.701	0.961	0.938	1												
9	-0.302	0.8	-0.996**	-0.017	0.403	-0.73	-0.54	-0.6	1											
10	-0.83	0.96	-0.75	-0.7	-0.133	-0.077	0.145	0.115	0.733	1										
11	0.815	-0.785	0.48	0.632	0.66	-0.148	-0.397	-0.118	-0.405	-0.64	1									
12	-0.009	0.586	-0.927	0.303	0.512	-0.892	-0.74	-0.833	0.931	0.45	-0.297	1								
13	-0.312	0.801	-0.985	0.002	0.281	-0.716	-0.507	-0.634	0.973	0.675	-0.535	0.953	1							
14	-0.364	0.126	0.155	-0.305	-0.827	0.404	0.517	0.197	-0.236	-0.071	-0.712	-0.155	-0.024	1						
15	-0.076	-0.52	0.905	-0.369	-0.632	0.934	0.81	0.852	-0.922	-0.414	0.16	-0.988	-0.914	0.299	1					
16	0.981*	-0.686	0.172	0.988	0.736	-0.591	-0.77	-0.678	-0.12	-0.737	0.741	0.182	-0.124	-0.381	-0.264	1				
17	0.875	-0.441	-0.119	0.983	0.777	-0.794	-0.903	-0.872	0.163	-0.544	0.539	0.469	0.181	-0.326	-0.53	0.951	1			
18	0.993**	-0.876	0.465	0.906	0.565	-0.317	-0.542	-0.432	-0.415	-0.892	0.821	-0.125	-0.419	-0.305	0.043	0.952	0.816	1		
19	0.838	-0.896	0.637	0.626	0.517	-0.018	-0.281	-0.036	-0.572	-0.771	0.978	-0.432	-0.669	-0.554	0.312	0.738	0.504	0.866	1	
20	0.749	-0.634	0.299	0.602	0.773	-0.269	-0.491	-0.186	-0.217	-0.473	0.977	-0.148	-0.377	-0.844	0.001	0.703	0.541	0.733	0.911	1

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at 0.01 level (2-tailed).

(1. Vigour index, 2. Shoot length, 3. Root length, 4. % Germination, 5. Conductivity, 6. Chlorophyll-*a*, 7. Chlorophyll-*b*, 8.Total chlorophyll, 9. Fresh weight, 10. Seed weight, 11. Leaf weight, 12. Root weight, 13. Reducing sugar, 14. Non reducing sugar, 15. Total carbohydrates, 16. Proteins, 17. DNA quantification, 18. DNA content, 19. DNA estimation, 20. RNA estimation)

correlated to reducing sugars. These observations indicate that clusters of 2 parameters were predominant in the present study.

In order to understand similarities between the various parameters, the data in Table 1 were also subjected to Bray-Curtis similarity index. A "fixed Stopping rule" at 90% similarity was applied. Chlorophyll-*a* and Chlorophyll-*b* were at closest distance while shoot length and fresh weight were the next closest clusters of similarity but at greater distances (Fig. 1). However, 80% and above similarity was represented by leaf weight and DNA content, conductivity and reducing sugars, root weight and fresh weight followed by higher similarity with conductivity and reducing sugars. These results point out that shoot length and fresh weight along with chlorophylls were the prominent parameters influenced by Tricyclazole in *Zea mays* (MHM 9001).

DISCUSSION

An increase in germination values compared to control may be specific response of *Z. mays* to the treatment of fungicide (Siddiqui 2004**)**. Though benlate caused an increase in chlorophyll, protein and carbohydrate contents of *Capsicum annum* and *Hibiscus esculentus* (Ahmed & Siddiqui 1995, Siddiqui et al. 1997), enhancement in germination as observed in the present study, has not been reported previously. The phenomenon also underscores the need for a comparative study of germination behaviour of different species in response to a particular fungicide. Increase in fresh and dry weight, root and shoot lengths of *Zea mays* (MHM 9001) were observed at 0.3% for root length, and in all the concentrations for shoot length. This may suggest a minimum threshold limit of fungicide that can be tolerated by the plants. Use of benlate has also been found to cause an increase in fresh and dry weights of *Sesbania sesban* at 0.25g/L concentration (Siddiqui et al. 1997). Siddiqui et al. (1999) also reported the inhibition of seed germination and seedling growth in *Pennisetum americanum* due to the application of organophosphate insecticides. Shive & Hugh (1976) showed that a fungicide triarimol not only prevented the gibberellin synthesis in *Phaseolus vulgaris* but also checked the dry weight increase over fresh weights at higher concentrations. The study suggests that a secondary toxicity mechanism non-operative at lesser concentration may become operative at higher concentration to cause a reduction in growth parameters like fresh and dry weights and root and shoot lengths. Windham & Windham (2004) have reported that systemic fungicides, which are based on SBI (sterol biosynthesis inhibitor), are closely related to plant growth regulators, the use of which at higher than labelled rates shorten the internodes leading to slow shoot growth. Use of high rates of systemic fungicides as seed treatments may also reduce the growth of small grains such as barley and wheat.

According to Pandey (1988), leakage as measured by conductivity is related to membrane disorganization. The lower the membrane integrity, the greater the electrolyte leakage in the steep water, thus, the greater the conductivity measurement (Powell et al. 1984). Woodstock et al. (1985) found relationship between weathering, deterioration, germination respiratory metabolism and leaching in cotton seeds. The deterioration of membranes due to weathering was confirmed by electron microscopy of cotyledon's lipids and proteins bodies and correlated well with conductivity measurements.

CONCLUSION

Treatment of *Zea mays* seeds with Tricyclazole influences root length and fresh weight and DNA significantly. The influence is more pronounced in the biochemical parameters like chlorophyll-*a* and chlorophyll-*b,* and morphological parameters like shoot length and fresh weight. A major effect of the treatment has an impact on root length which in turn may have an influence on the morphological and biochemical parameters.

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