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Effect of a Systemic Fungicide Metalaxyl on Seed Germination, Seedling Growth and Chlorophyll Content in Maize (*Zea mays* L)

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Abstract: ***The present study was conducted to investigate the effect of metalaxyl, a systemic fungicide on the morphological and physiological in maize during early germination. The result showed a decrease in germination percentage, shoot length, fresh weight and vigor index in all the concentrations of fungicide treated except for 4.5mg treatment which was at par with the control. Chlorophyll content found to be decreased over control and Electrical conductivity increased with increasing concentration of metalaxyl. The above result indicates 7mg concentration of metalaxyl proves to be phyto toxic to the maize seedlings whereas 4.5mg has a slight stimulatory effect.***

Keywords: metalaxyl, systemic fungicide, seed germination, chlorophyll and electrical conductivity

1. Introduction

Agricultural crops are continuously exposed to various biotic and abiotic stresses which reduce their yield and productivity (1). Biotic stress mainly due to disease causing pathogens also reduces the yield. But plants have evolved various mechanisms such as changes in the physiological, biochemical, molecular and genetic level for coping with such environmental stress conditions (2). Maize is susceptible to many fungal pathogens, pathogenic bacteria, nematodes and viruses and also to mycoplasma like organisms and one parasitic like higher plant (3). Use of fungicides performs different roles as, it controls the disease caused by fungi during early stage of growth and development of a crop plant, enhances the productivity or yield of the crop and also improves the shelf life of the harvested crops during storage (4). Therefore, the present study was undertaken to know the effect of metalaxyl, a systemic fungicide on percentage of seed germination, vigor index, chlorophyll a, chlorophyll b, Totalchlorophyll, conductance, and phenolic contents in maize.

2. Literature Survey

Seed dressing with fungicide is a method used for the control of seed borne & soil borne pathogens that results in healthy development of plants which in turn results in increased crop yield (5). Although the seed treatments protect the seed & seedlings from pests & disease, it causes secondary effects on germination & growth.

3. Materials and methods

Maize seeds were procured from V. C. Farm, Zonal Agricultural Station, Mandya, Karnataka, India. 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 metalaxyl. Dose range of fungicide was selected depending on the prescribed concentration that could affect 10-95% of the seedlings with logarithmic intervals. Germination of maize grains was completely inhibited above 8mg/gm of metalaxyl. Hence, maize Seeds were treated with concentration below 8mg/gm i.e. 1.5mg, 3mg, 4.5mg, 6mg and 7mg/gm. The first and second count of germination percentage was taken on 4th and 7th day as prescribed by ISTA (6). Various parameters like seed germination, root length, shoot length, fresh weight, dry weight, vigor index, chlorophyll content and conductance were analyzed.

3.1 Germination percentage

Radicle emergence of 1 mm was taken as germinated and germination percentage was calculated by using the formula prescribed by ISTA (7).

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} x100$$

3.2 Root length and shoot length

The root and shoot lengths were measured using a ruler. The root length was measured from the distance between collar and tip of root and expressed incentimeter. The shoot length was measured from the distance between collar and tip of shoot and expressed in centimeter. 4th and 7th day germinated seedlings were used to measure the length of root and shoot.

3.3 Seed Vigour Index (SVI)

Vigour index of the seedling was calculated by using the formula suggested by Abdul-Baki and Anderson (8).
SVI = (Seedling length) × Germination percentage
Where,
Seedling length = Mean root length + Mean shoot length

3.4 Fresh and Dry weight of seedlings

The ten seedlings of 7th day germination were collected and weighed to determine the fresh weight. The seedlings were later dried in hot air oven at 80°C for 48 hours to determine their dry weight which was carried out in an electrical single pan balance. The procedure was repeated using three replicates and the total seedling weight was calculated by dividing the value by 10 according to ISTA (6).

3.5 Conductivity studies

The conductivity of seed lechate was estimated using the method suggested by Mullet and Wilkinson (9). The conductivity measurements were made using HacCDC-401 conductivity meter.

3.6 Estimation of chlorophyll

The pigments, chlorophyll – a, chlorophyll – b, total chlorophyll was estimated as per the method of Arnon (10). The amount of chlorophyll was calculated and expressed as mg/g of fresh weight.

3.7 Statistical Analysis

The data collected were subjected to analysis of variance and the means were compared according to Tukeys's (11) significance test at 5% level using computerized SPSS package version 13.0. There are significant differences between the means of four doses when the probability value (p) is less than or equal to the risk $\alpha = 0.05$ ($p \leq \alpha = 0.05$); highly significant differences when $p \leq \alpha = 0.01$.

4. Results and Discussion

4.1 Effect of metalaxyl on percent germination

The result of mean percent germination of maize seedlings on 4th and 7th day of germination treated with different concentrations of the fungicide is presented in Table 1. The result showed a decrease in germination percentage in maize seedlings with increase in fungicide concentrations ranging from 1.5 to 7mg however 4.5mg treated seedlings did not show much variation in germination percentage. The result is in agreement with the findings of Monika *et al.,* (12). Many studies have 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). The slight reduction in germination percentage observed in metalaxyl treated seeds over control in the present study may be due to the impaired mobilization and/or utilization of seed reserves.

4.2 Root length and shoot length

In the present study there was significant increase in root length with increase in concentration of metalaxyl observed from 4th -7th day of germination (Table 2 and Table 3). These findings are in agreement with Anitha and Savitha (15) have reported increase in root length with increasing concentration of carbendazim in the rice cultivar Jyothi. Similarly,carbendazim treated wheat seedlings showed increased root length compared to untreated seedlings (16, 17). Increase in the cytokinin and abscisic acid and inhibition of gibberlic acid level as observed in triazole treated *C.roseus* seedlings by Jaleel *et al*., (18) may be one of the reason for the increased root length in metalaxyl treated maize seedlings.

There was a significant decrease in shoot length with different concentrations of fungicide treatment compared to the control (Table 3). Windham and Windham (19) have opined that the systemic fungicides which are related to sterol biosynthesis inhibitor act as plant growth regulator and use of these fungicides at higher concentrations may shorten the internode, thus leads to reduction in the shoot growth of plants. Alla *et al.,* (20) opined that the growth reduction might be due to altered nitrogen metabolism in wheat and maize plants treated with herbicides. The decrease in the shoot length observed in the present study when treated with metalaxyl may be due to shortening of internodes which results in reduced shoot growth.

4.3 Seed Vigour index

As observed in Table 4, a decreased vigour index in treated seedlings is seen compared to control on all the days of study and the highest decrease was observed in 7mg treated seedlings. A decline in seedling vigour with increase in salinity stress was reported in two cultivars of chickpea (21) and in haricot bean under copper sulphate treatment (22).

4.4 Fresh weight and dry weight of Seedlings

The present study showed a reduction in fresh weight (Table 5) except in 4.5mg treated seedlings and increase in the dry weight of maize seedlings (Table 6) in the metalaxyl treated seedlings when compared with the control. Reduction in the fresh and dry weight of all the varieties of rice was observed in salt stress (23). A study by Mahendiran *et al.,* (24), have reported enhancement in the fresh and dry root and shoot weight in rice varieties treated with thiamethoxam. Reduction in the fresh weight of metalaxyl treated maize seedlings compared to control observed may be a specific response of the maize seedlings under the fungicide treatment whereas an increase in the dry weight can be attributed to the role of abscisic acid in plant growth (25).

4.5 Conductivity studies

The electrical conductivity increased after soaking the seeds for 24hours in different concentrations of fungicide. This result (Table 7) is very well correlated with vigour index of the present investigation. A low vigour index and high conductivity values are observed at 7mg metalaxyl treated seedlings. Similar correlations can be made with the other treatments also. This indicates 7mg concentration of metalaxyl proves to be phyto toxic to the maize seedlings.

4.6 Chlorophyll pigment

The chlorophyll-a, chlorophyll-b and total chlorophyll content decreased over control in all the days of observations when treated with different concentrations of metalaxyl in maize seedlings ((Table 8, 9, 10). The decrease

in pigment content was found to be significant between the control and the treatments of maize seedlings, but it was not significant between the treatments.

In the present study, decreased chlorophyll content may be due to degradation of chlorophyll pigments or increased activity of chlorophyllase which can inhibit chlorophyll synthesis under the fungicide stress.

5. Conclusion

The data obtained suggests that all the morphological and physiological parameters studied were affected above and below 4.5mg/g concentration of metalaxyl whereas 4.5mg/g 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 and also an inappropriate use of fungicide in crop production without the proper understanding of the fungicides– plant interactions is to be avoided so that the yield of crops can be protected from the toxicity of fungicides.

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Table 1: Effect of different concentrations of metalaxyl on percent germination of maize seedlings

Germination in days	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3.0	4.5	6.0	7.0	
4th day	91.2^{c}	90.4^{c}	81.7^{b}	90.8^{c}	72.6^{a}	70.7^{a}	82.90^{a}
7th day	97.1^{c}	94.5^{c}	83.1^{b}	96.6^{c}	79.2^{a}	76.4^{a}	87.65^{b}
Mean	94.15^{d}	92.4^{d}	82.4^{c}	93.7^{d}	75.9^{b}	73.5^{a}	85.27
F value	Concentrations F (5,24) = 445.235**, Days F (1,24) =176.576** Concentration * Days = F (5, 24) = 4.512**						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at P ≤ 0.05

Table 2: Effect of different concentrations of metalaxyl on root length (cm) of maize seedlings

Germination in days	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3	4.5	6	7	
4th day	7.13^{a}	8.15^{a}	8.32^{a}	9.21^{a}	6.96^{a}	8.49^{a}	8.02^{a}
7th day	10.78^{a}	11.13^{ab}	11.25^{a}	14.67^{b}	11.35^{a}	12.84^{ab}	12.08^{b}
Mean	8.95^{a}	10.17^{ab}	9.78^{a}	11.86^{b}	8.94^{a}	10.59^{ab}	10.05
F value	Concentrations F (5,24) = 6.366**, Days F (1,24) =129.073** Concentration * Days = F (5, 24) = 0.802						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at ≤ 0.05

Table 3: Effect of different concentrations of metalaxyl on shoot length (cm) of maize seedlings

Germination in days	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3	4.5	6	7	
4th day	4.12^{a}	3.42^{a}	3.76^{a}	4.35^{a}	2.73^{a}	1.57^{a}	3.35^{a}
7th day	6.75^{a}	5.11^{a}	5.48^{a}	6.59^{a}	4.81^{a}	4.98^{a}	5.62^{b}
Mean	5.43^{b}	4.28 ^{ab}	4.61^{ab}	5.48^{b}	3.72^{ab}	3.35^{a}	4.49
F value	Concentrations F (5,24) = 3.908**, Days F (1,24) =40.412** Concentration * Days = F (5, 24) = 0.39						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at ≤ 0.05

Table 4: Effect of different concentrations of metalaxyl on vigour index of maize seedlings

Germination in days	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3	4.5	6	7	
4th day	382.85^{d}	317.31^{c}	315.51^{c}	404.19^{d}	205.16^{b}	119.49^{a}	291.01^{a}
7th day	666.20^{d}	494.02^{c}	466.64b^{c}	651.26^{d}	392.30^{a}	393.31^{ab}	513.64^{b}
Mean	522.76^{d}	406.21^{c}	393.05^{c}	530.84^{d}	301.49^{b}	259.59^{a}	402.32
F value	Concentrations F (5,24) = 144.991**, Days F (1,24) = 874.120** Concentration * Days = F (5, 24) = 8.815**						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at ≤ 0.05

Table 5: Effect of different concentrations of metalaxyl on fresh weight (g) of maize seedlings

Germination in days	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3.0	4.5	6.0	7.0	
0 day	7.201^{a}	7.187^{a}	7.153^{a}	7.213^{a}	7.267^{a}	7.282^{a}	7.231^{a}
4th day	7.371^{a}	7.389^{a}	07.34^{a}	7.372^{a}	7.455^{a}	7.476^{a}	7.451^{b}
7th day	8.93^{b}	8.74^{ab}	8.67^{ab}	8.88^{ab}	8.73^{ab}	8.31^{a}	8.698^{c}
Mean	7.824^{a}	7.764^{a}	7.721^{a}	7.874^{a}	7.877^{a}	7.695^{a}	7.793
F	Concentrations F (5,36) = 1.094, Days F (2,36) = 221.783** Concentration * Days = F (10, 36) = 1.248						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at ≤ 0.05

Table 6: Effect of different concentrations of metalaxyl on dry weight (g) of maize

Germination In days	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3.0	4.5	6.0	7.0	
0 day	2.31^{a}	2.39^{a}	2.49^{a}	2.20^{a}	2.34^{a}	2.46a	2.422^{a}
4^{th} day	2.46^{a}	2.52^{a}	2.65^{a}	2.76^{a}	2.84^{a}	2.97^{a}	2.611^{a}
7^{th} day	2.874^{ab}	2.902^{ab}	3.02^{b}	2.709^{a}	2.816^{ab}	2.816^{ab}	2.852^{b}
Mean	2.554^{a}	2.605a	2.730^{a}	2.503^{a}	2.653^{a}	2.724^{a}	2.628
F	Concentrations F (5,36) = 1.232, Days F (2,36) = 13.726** Concentration * Days = F (10, 36) = 0.574						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at ≤ 0.05

Table 7: Effect of metalaxyl on conductivity (µmho^{-1}cm^{-1}g seed) of maize seeds

After 24 hours of imbibition	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3	4.5	6	7	
	216^{a}	268^{a}	305^{ab}	258^{a}	482^{bc}	521^{c}	341.66
F value	Concentrations F (5,12) = 9.933**						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at P ≤ 0.05

Table 8: Effect of metalaxyl on the chlorophyll- a (mg/g fresh wt.) in the germinating maize seedlings

Germination in days	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3	4.5	6	7	
4	0.552^{c}	0.249^{b}	0.232^{b}	0.185^{ab}	0.167^{ab}	0.11^{a}	0.250^{a}
5	0.651^{e}	0.552^{d}	0.482^{c}	0.226^{b}	0.173^{a}	0.207^{b}	0.382^{b}
6	0.953^{e}	0.924^{d}	0.901^{cd}	0.876^{c}	0.762^{b}	0.602^{a}	0.836^{d}
7	1.1^{d}	0.77^{c}	0.691^{bc}	0.602^{bc}	0.504^{b}	0.29^{a}	0.660^{c}
Mean	0.814^{e}	0.625^{d}	0.577^{d}	0.472^{c}	0.402^{b}	0.302^{a}	0.531
F value	Concentrations F (5,48) = 224.630**, Days F (3,48) =724.445** Concentration * Days = F (15, 48) = 15.667**						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at P ≤ 0.05

Table 9: Effect of metalaxyl on the chlorophyll – b (mg/g fresh wt.) in the germinating maize seedlings

Germination in days	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3	4.5	6	7	
4	0.23^{b}	0.172^{ab}	0.141^{ab}	0.083^{a}	0.074^{a}	0.07^{a}	0.128^{b}
5	0.324^{d}	0.241^{c}	0.161^{b}	0.151^{b}	0.162^{b}	0.123^{a}	0.193^{c}
6	0.029^{a}	0.025^{a}	0.021^{a}	0.025^{a}	0.017^{a}	0.02^{a}	0.022^{a}
7	0.34^{b}	0.31^{b}	0.263^{ab}	0.254^{ab}	0.249^{ab}	0.11^{a}	0.254^{d}
Mean	0.230^{d}	0.187^{cd}	0.146^{bc}	0.128^{ab}	0.125^{ab}	0.080^{a}	0.150
F	Concentrations F (5,48) = 18.885**, Days F (3,48) =100.913** Concentration * Days = F (15, 48) = 2.892**						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at P ≤ 0.05

Table10: Effect of metalaxyl on the total chlorophyll (mg/g fresh wt.) in the germinating maize seedlings

Germination in days	Control	Concentration of metalaxyl (mg/g)					Mean
		1.5	3	4.5	6	7	
4	0.792^{e}	0.432^{d}	0.367^{c}	0.272^{b}	0.246^{b}	0.122^{a}	0.372^{a}
5	1.013^{e}	0.802^{d}	0.767^{c}	0.453^{b}	0.427^{b}	0.339^{a}	0.634^{b}
6	1.24^{d}	1.12^{cd}	1.01^{bc}	0.934^{ab}	0.839^{a}	0.805^{a}	0.992^{c}
7	1.35^{e}	1.23^{de}	1.1^{cd}	0.949^{bc}	0.763^{b}	0.43^{a}	0.970^{c}
Mean	1.09^{f}	0.896^{e}	0.812^{d}	0.652^{c}	0.569^{b}	0.424^{a}	0.742
F value	Concentrations F (5,48) = 274.55 **, Days F (3,48) = 612.371** Concentration * Days = F (15, 48) = 11.164**						

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, according to Tukey's mean range test at 5% level. ** Significant at P ≤ 0.01, *significant at P ≤ 0.05