***Original Research Article***

# Metalaxyl induced changes in Protein metabolism during germination of Maize


## Abstract

The present work was carried out to investigate the effect of fungicide, metalaxyl as a seed treatment on the protein metabolism of maize during early germination. The study was carried out for 7 days after soaking the seeds in different concentrations of metalaxyl and a control was maintained. Biochemical analyses of whole seedlings were done up to 7 days. Changes in the amount of total soluble protein, total free amino acids, and the protease activity were performed using standard methods. The results indicate that protein content was maximum on $4^{th}$ and $6^{th}$ day of germination in both control and treated seeds. Metalaxyl treatment resulted in the decreased protein content (20-50% inhibition) over the untreated in a dose dependent manner till the $4^{th}$ day of germination. But as the growth proceeded a proportionate increase in the protein content was observed on the $6^{th}$ day of germination in fungicide treated seeds compared to the control. The specific activity of protease was decreased by 46%, 81%, 88%, 97%, and 100% with 1.5mg, 3mg, 4.5mg, 6mg and 7mg concentration of Metalaxyl on $3^{rd}$ day of germination when compared to the control. An increase in the total free amino acids occurred during the germination and maximum free amino acids content was observed on the $5^{th}$ day. While, Metalaxyl treatment resulted in the dose dependent depletion of free amino acids content. A significant increase in proline content was found to occur in treated seeds. From the overall findings, the present study gives an insight into a protective effect of the system with an increased production of proline and decreased protease activity, free amino acids and at the same time a higher protein content during later stages of germination for a particular concentration which may be due to the synthesis of novel proteins as a defense mechanism indicating the dual role of metalaxyl.


*Keywords: Metalaxyl; germination; protease; proteins; free amino acids; proline.*

## 1. INTRODUCTION

The mobilization of seed storage proteins represents one of the most important post-germination events in the growth and development of seedling. Proteolytic enzymes play a central role in the biochemical mechanism of germination [1]. During germination period, the storage proteins are degraded by a variety of proteases which convert the insoluble storage proteins in to soluble peptides and these peptides are further hydrolyzed to free amino acids. These free amino acids are mobilized to the embryonic axis to support its growth and also to provide energy [2]. Downey mildew of maize (*Zea mays.L)* caused by *Peronosclerospora* is one of the most destructive diseases of this crop in the tropical Asian countries [3]. Integrated approaches are used to manage the disease including crop rotation, planting resistant cultivars and the application of fungicides like mancozeb and Metalaxyl. Metalaxyl compounds have been widely used for the control of Downey mildew in a number of crops. Metalaxyl (methyl *N*-(methoxyacetyl)-*N*-(2, 6-

xylyl)-DL-alaninate) is a systemic fungicide acts by suppressing sporangial formation, mycelia growth and establishment of new infection [4]. Much of our knowledge of reserve mobilization and its control processes during germination is very well understood. In contrast, available information on metalaxyl is limited to its effect on the pathogen and not much data is available on the effect of metalaxyl in protein metabolism of maize seeds. Hence, the present work is an attempt for furthering our knowledge towards better understanding of the effect of metalaxyl in germinating maize seeds focusing on the protein metabolic changes during germination.

## 2. MATERIAL AND METHODS

### 2.1 Collection of seeds and Treatments

Maize seeds were procured from VC farm, University of Agriculture Science, Mandya, Karnataka. All the chemicals were purchased from SLR and MERCK and the chemicals were of analytical grade. Seeds were surface sterilized with 0.1% mercuric chloride for 10 minutes and repeatedly washed with distilled water for 4-5 times. Seeds of uniform size were selected and soaked for 24 hours in distilled water (control)and with different concentrations(mg/g) of metalaxyl, 1.5, 3, 4.5, 6 and 7mg/gm of the seeds(1:5 weight/volume) for 24 hours. Five seeds in triplicate were placed on petridish with 8-10 layer of soaked filter paper and incubated at $25^0$c both in light and dark condition. Uniform seedlings were selected and processed for further studies. Everyday filter paper was wetted with 10ml of distilled water. [5]

### 2.2 Preparation of crude extract

Around 1-2 gram of seedling treated with different concentrations of the metalaxyl and the untreated seeds were taken each day, (up to 7 days) homogenized in ice cold saline (5ml) using pestle and mortar. The solution was centrifuged for 10 minutes and supernatant were used for further analysis.

### 2.3 Biochemical studies

Protein was estimated as described by Lowry et al. [6] using BSA as standard. The activity of Protease was determined following the procedure of Kunitz [7]. Total free amino acids were extracted and determined following the method of Sugano et al [8].Free proline content was estimated following the method of Bates et al [9].

### 2.4 Statistical Analysis

The data are expressed as the mean ± SEM analyzed by one-way analysis of variance (ANOVA) and Dunnett's *t*-test was used as the test of significance. P value < 0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS statistical software.

## 3. RESULTS AND DISCUSSION

### 3.1 Total Protein

The protein content during different days of germination in control and fungicide treated seedlings is presented in Table-1. The protein content was maximum on $4^{th}$ and $6^{th}$ day of germination in both control and treated seeds. Metalaxyl treatment resulted in the decreased protein content (20-50% inhibition) over the untreated in a dose dependent manner till the $4^{th}$ day of germination. The treated seeds showed similar pattern of protein content on different days of germination. However an increase in the protein content was seen on the $6^{th}$ day of germination in treated seeds.

**Table 1- Effect of Metalaxyl on the Total Protein (mg/g) in the seedlings of Maize.**

| Concentration of Metalaxyl (mg/g) | Seedling age in days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | 12.6 ±0.9 | 24.2±1 | 26.69±1.4 | 10.75±0.6 | 42.48±2.1 | 29.52±1.4 | 57±3.1 | 50.65±1 |
| 1.5 | 10.7±0.7 | 22.82±1 | 24.45±1.5 | 9.21±0.3 | 34.09±1.4 | 21.54±1.2 | 76.4±2 | 60.49±1.9 |
| 3.0 | 13.1±0.5 | 25.43±1.2 | 25.91±1.2 | 9.71±0.4 | 30.8±1.2 | 33.15±1.5 | 96.06±2.4 | 31.96±1.1 |
| 4.5 | 34.4±1.2 | 27.61±1 | 25.69±0.4 | 2.71±0.1 | 26.78±0.9 | 33.69±1.6 | 67.51±2.3 | 52.4±2.1 |
| 6.0 | 12.6±1 | 25.18±1.1 | 12.43±0.3 | 12.14±0.6 | 20.16±0.5 | 17.87±0.9 | 63.19±2.3 | 22.33±0.8 |
| 7.0 | 10±0.5 | 21.12±0.9 | 23.15±1.1 | 7.65±0.3 | 25.45±0.7 | 15.91±0.7 | 47.52±1.2 | 31.21±0.9 |

In the present study the protein content in germinated seeds were found to be increased ,due to the mobilization of storage nitrogen for the production of protein needed for the development of the young seedling and the observation was well in agreement with the studies conducted in germinated seeds [10]. A decline in the protein content was observed in treated seeds till the 4$^{th}$ day in a dose dependent fashion. The decrease in the protein content in fungicide treated maize may be due to osmotic shock effect by the fungicides which results in the release of protein and loss of membrane transport ability [11]. It has been suggested that the toxicant produced in the treated seeds may inhibit the protein synthesis by binding to the larger ribosomal subunit inducing change in the enzyme system ceasing ATP and NADP formation .The results of our studies were in parallel, as the concentration of fungicide increases, the amount of protein gradually decreased. But as the growth proceeded a proportionate increase in the protein content was observed on the 6$^{th}$ day of germination in fungicide treated seeds compared to the control. The enhanced protein content may be attributed to the fact that as growth proceeds, Metalaxyl may induce the expression of many defense related genes in plants which results in the synthesis of novel proteins. The results of our study correlates with the recent proteomic analysis which revealed the occurrence of novel proteins in several plant species under heavy metal stress [12].

## 3.2 Activity of Protease

Protease is a hydrolytic enzyme which acts on proteinaceous substance to produce amino acid and amides. The specific activities of protease during germination of maize seeds treated with Metalaxyl are shown in Fig 1. Both control and treated seeds showed increasing specific activity up to 3$^{rd}$ day of germination and decreased then onwards. Specific activity of protease in maize seedling at different concentration of Metalaxyl on 3$^{rd}$ day were found to be 27, 9.1, 6, 1.6 and 0.3 $\times 10^{-7}$mM whereas in control the activity is found to be $50 \times 10^{-7}$ mM . The specific activity was decreased by 46%, 81%, 88%, 97%, and 100% with 1.5mg, 3mg, 4.5mg, 6mg and 7mg concentration of Metalaxyl on 3rd day of germination when compared to the control.

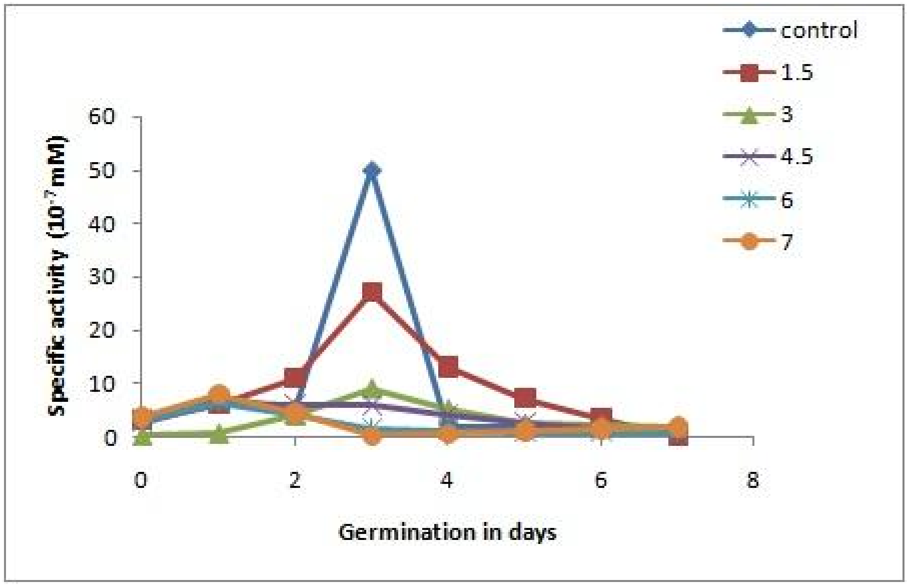


**Fig.1. Effect of Metalaxyl on the *Specific activity of Protease in the seedlings of Maize**

**Specific activity of Protease is expressed as $10^{-7}$mM of proteins degraded/mg of protein/min*

Increasing in proteolytic activity with concomitant reserve protein depletion agrees with the findings of earlier studies on other seeds; *Phaseolus vulgaris* [13], *Lupinus albus* [14] *Vicia sativa* [15] and *Macrotyloma uniflorum* [16].The result from our study is not parallel with the above data as there is a dose dependent inhibition of protease activity in Metalaxyl treated maize seeds on 3rd day of germination. Impairment of proteasome functionality and decreased protease activities seems to be a common feature involved in metal toxicity in plants [17].

### 3.3 Free amino acid

The amino acid content in control and treated seeds during different days of germination is presented in the Fig.2. A gradual increase in the amino acid content is seen and the maximum content of amino acid ($41 \times 10^{-1}$) was seen on the 5th day in control seed and a gradual decrease was seen till the 7th day of germination. The free amino acid content was decreased effectively with the increasing concentration of fungicides with the maximum inhibition was seen with the highest concentration (7mg/g). Only 30% of free amino acids were present in that concentration. An increase in the total free amino acids occurred during the germination and maximum free amino acids content was observed on the 5th day While, metalaxyl treatment resulted in the dose dependent depletion of free amino acids content. There was no change in the pattern of amino acid profile on different days of germination in both treated and control seeds. These result suggests, the protective effect of amino acids against unfavorable condition, increasing the tendency of the system to maintain the homeostasis.

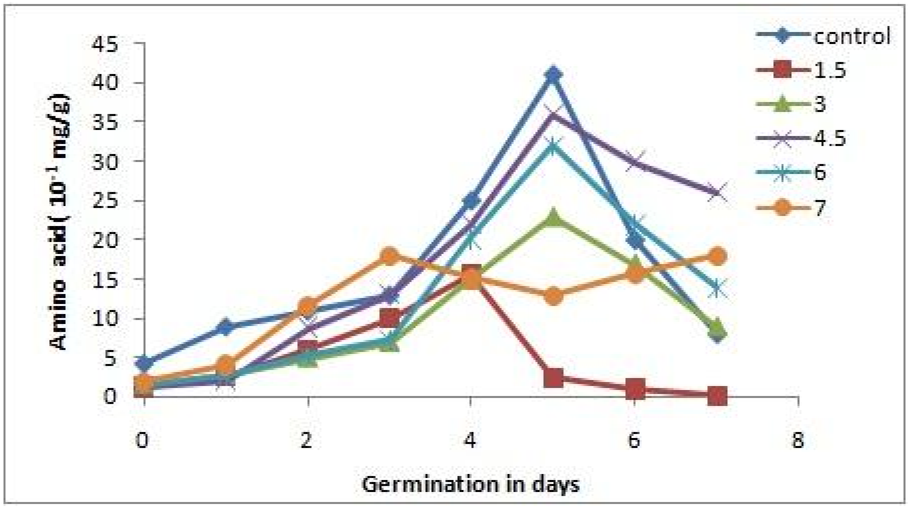


**Fig.2. Effect of Metalaxyl on the Amino acid content ($10^{-1}$ mg/g) in the seedlings of Maize**

### 3.4 Proline content

Proline content in control and treated seeds are presented in the Fig.3.The proline content increased in treated seeds and maximum proline content was seen with 3mg/g and in 6mg/g concentration of Metalaxyl (0.797mg/g, 0.705mg) on 5$^{th}$ day of germination and decreased thereafter. The proline content was lowest in control (0.043mg/g) on 5$^{th}$ day and in treated (7mg/g) and it was 0.06mg/g on 7$^{th}$ day. A significant increase in proline content was found to occur in treated seeds.

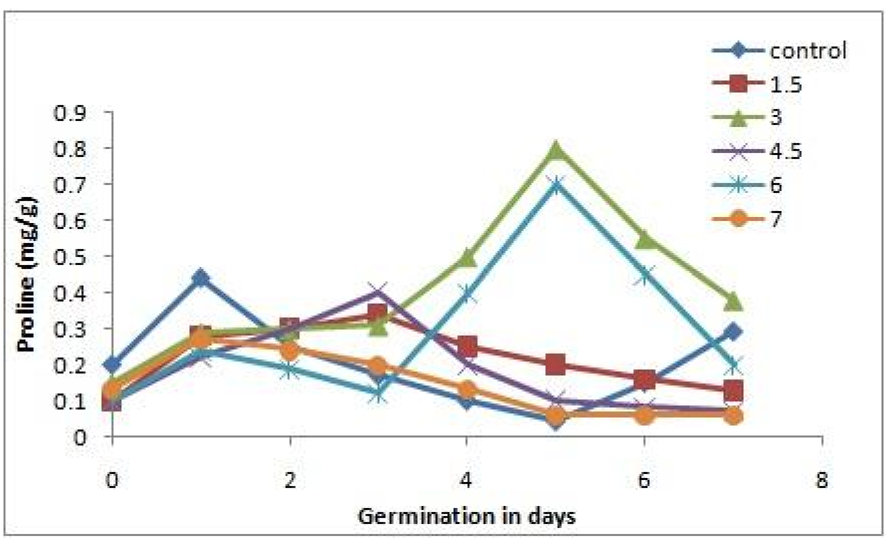


**Fig.3. Effect of Metalaxyl on the Proline content (mg/g)) in the seedlings of Maize**

Proline is considered as an antioxidant free radical scavenging and biochemical indicator of stress. It is considered as a metabolic measures of abiotic stress [18].In the present work the stress caused by metalaxyl resulted in an increase in proline level. In general water stress has been known to increase free proline in leaves[19].Plant growing in stress condition need to produce specific proteins having higher proline content (hydoxyproline rich glycopeptide or proline rich glycopeptides) [20].The high content of free proline inhibits proline biosynthesis in plants growing under heavy metal contamination [21].Effect of this change is subsequently contributed to higher production of glutamic acid which stressed plants need to create phytochelatins[22].

## 4. CONCLUSION

From the overall findings, the present study gives an insight into a protective effect of the system with an increased production of proline and decreased protease activity, free amino acids and at the same time a higher protein content during later stages of germination for a particular concentration which may be due to the synthesis of novel proteins as a defense mechanism indicating the dual role of metalaxyl. The biosynthesis of these proteins depends on the supply of free amino acids. This is correlated with the fact that there is a depletion of free amino acids in a dose dependant manner. Proline which is considered to be the biochemical indicator of stress, found to increase significantly in metalaxyl treated seeds. This study has helped us in understanding the changes in protein metabolism during germination process in control and in the fungicide treatment. Further work is in progress in investigating the increased level of protein in maize during germination in treated with different concentrations, which may help us in furthering our knowledge on the mechanism and its regulation.

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