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# Effect of Guignardia toxins on germination of seeds & on activities of amylolytic enzymes

**A. H. Ansari**

Associate Professor

Department of Biochemistry, S.K. Porwal College, Kamptee,

Corresponding author: Email – ahfazulhaqueansari@gmail.com

## Abstract

Several toxins elaborated by plant pathogens, including **Guignardiacitricapa Kiely**, produce inhibitory effects on germination & growth of seedlings. They also known to inhibit activities of different enzymes in germinating seedlings. The most suitable medium for growth & toxin yield of **Guignardiacitricarpa Kiely**, the Czapek's – Dox rice medium, was inoculated & incubated at $30^0$C ± 2 for 28 days. The medium was then air dried. The toxins from the dried medium were extracted with ethyl acetate & evaporated to dryness. The test solution was prepared by dissolving a suitable amount of toxins in distilled water containing few drops of tween 80. Experiments were performed to observe the effect of **Guignardia** toxins on germination & growth of various seedlings viz:**Triticum vulgare** (Wheat), **Brassica juncea** (Mustard), **Cyamopsis tetragonoloba** (Cluster bean), **Zea-mays** (Corn), **Hibiscus esculentus** (Lady's finger) &**Phaseolus mungo** (Moong bean). The effect on activities of amylolytic enzymes viz: amylase & invertase was also investigated in **Brassica** seedlings.

**Guignardia** toxins showed a considerable reduction in germination & growth of seedlings tested. Remarkable increase in activitiesof amylase & invertase was noticed in toxin treated germinating **Brassica** seedlings.

Key Words: Germination of seeds, amylase, invertase, **Guignardiacitricarpa Kiely**.

## Introduction

A number of toxins produced by plant pathogens are known to inhibit the germination & growth of seedlings [6, 10]. During germination metabolic changes occurring in early stages are a result of the activity of various enzymes which may either be present in dry seeds or be synthesized as germination proceeds. Several workers have observed inhibitory effect of toxins on activities of different enzymes as well as on protein synthesis in germinating seedlings [7, 8]. Thus, it appears to be a considerable effect of the toxins on germination of seeds & other process.

In view of these observations, it was thought worthwhile to observe the effect of toxins, isolated from the culture extract of **Guignardiacitricarpa Kiely**, a black spot pathogen of citrus fruit, on germination in seedlings viz: **Triticum vulgare** (Wheat), **Brassica juncea** (Mustard), **Cyamopsis tetragonoloba** (Cluster bean), **Zea-mays** (Corn), **Hibiscus esculentus** (Lady's finger) &**Phaseolus mungo** (Moong bean). The organism, first reported by Kiely [1],is included under "Ascomycetes", a higher class of fungi with about 35,000 species [2]. Inaddition,the effect on the activities of some amylolytic enzymes viz: amylase & invertase in germinating Brassica seedlings was also investigated.

# Materials & Methods

**Isolation & Separation of toxins**

Czapek's – Dox rice medium, found to give maximum growth & toxin yield [4, 5], was inoculated & incubated at $30^0$C ± 2 for 28 days. After incubation the medium was air dried [4] & toxins were extracted from the dried medium with ethyl acetate [3]. The extract was filtered & evaporated to dryness. 150 mg of toxins dissolved in 10 ml of distilled water containing 2 to 3 drops of tween 80 was prepared & used as test solution.

Seeds of uniform size of **Triticum vulgare** (Wheat), **Brassica juncea** (Mustard), **Cyamopsis tetragonoloba** (Cluster bean), **Zea-mays** (Corn), **Hibiscus esculentus** (Lady's finger) &**Phaseolus mungo** (Moong bean), selected to study the effect of toxins on germination, were surface sterilized by 1 % $HgCl_2$ solution for 2 minutes & rinsed repeatedly in sterile distilled water. They were then placed (15 seeds/plate) on double layer of paper toweling of Whatman No. 1 filter paper in previously sterilized petri plates [9] & 3 to 4 ml of test solution was added to each of them. Control plates were also run simultaneously in which distilled water containing 2 to 3 drops of tween 80 was added. These plates were covered & placed in humid dark germination chamber (maintained at $30^0$C) for 48, 72, 96 120 hours. The number of seeds germinated in each case was measured at regular intervals. Seeds from which radicle of 2 mm size emerged were considered as germinated [10].

**Growth of the seedlings**

The growth of seedlings was measured & expressed in cm after 48, 72, 96 & 120 hours by measuring the length of the radicle & hypocotyl as per the case [9].

**Enzyme assay**

Activities of enzymes were measured in germinated **Brassica** seedlings after 48, 72, 96 & 120 hours of germination. For measuring enzymeactivities, 10 % extract was prepared. One gram of the material was crushed in a mortar using cold distilled water with sterilized sand [11] & made up to volume of 10 ml. Homogenate was then clarified by centrifugation at 12,000 x g for 20 minutes. The supernatant was used as enzyme preparation.

Amylase & invertase activities were measured following the methods of Mattoo& Modi [12] & Hatch &Glasziou [11] respectively. Reducing sugar liberated was determined by Nelson's method [13]. Appropriate zero-time controls were run simultaneously. One unit of enzyme activity, in both the cases, was defined as that amount of enzyme which liberates 1 mg reducing sugars per 30 minutes at $37^0$C.

Specific activity was expressed as units per mg protein. Proteins in enzyme preparations were estimated by the method of Lowry et al [14].

# Results & Observations

In case of all the seeds tested, a considerable inhibition of germination was observed at the given concentration of toxin as compared to control.

**Growth of the radicle & hypocotyl**

During the first 48 hours, a considerable inhibitory effect of the **Guignardia** toxins was noticed in the length of radicle & hypocotyl in all the germinated seedlings as compared to control groups

(Table: 1). The inhibitory effect of the toxins was also observed in the formation of leaves in case of **Brassica juncea**.

Activities of both the enzymes viz: amylase & invertase, have shown increased activity in toxin treated seeds as compared to controls. Changes in activities of these enzymes studied in **Brassica juncea** seedlings are shown in Tables: 2 & 3 (Figures: 1 & 2).

**Table: 1**

**Effects of Guignardiacitricarpa toxins on length of radicle & hypocotyl on germination of seedlings**

| S. No. | Seedlings | | Hours of Germination | | | |
|---|---|---|---|---|---|---|
| | | | 48 | 72 | 96 | 120 |
| 1. | Tritium vulgare (Wheat) | Radical | C – 1.1<br>E – 0.8 | C – 2.0<br>E – 1.6 | C – 3.8<br>E – 2.4 | C – 4.2<br>E – 2.7 |
| | | Hypocotyl | C – 0.9<br>E – 0.7 | C – 2.4<br>E – 1.3 | C – 3.9<br>E – 2.6 | C – 4.4<br>E – 2.6 |
| 2. | Brassica juncea (Mustard) | Radical | C – 0.9<br>E – 0.6 | C – 2.0<br>E – 1.3 | C – 3.4<br>E – 2.0 | C – 3.8<br>E – 2.2 |
| | | Hypocotyl | C – 0.3<br>E – 0.2 | C – 1.8<br>E – 1.0 | C – 3.1<br>E – 1.8 | C – 3.4<br>E – 2.0 |
| 3. | Cyamopsis Tetragonoloba (Cluster bean) | Radical | C – 1.0<br>E – 0.6 | C – 1.9<br>E – 1.2 | C – 2.4<br>E – 1.6 | C – 3.5<br>E – 2.9 |
| | | Hypocotyl | C – 0.3<br>E – 0.2 | C – 1.4<br>E – 0.9 | C – 2.6<br>E – 1.5 | C – 4.8<br>E – 2.0 |
| 4. | Zea-mays (Corn) | Radical | C – 0.6<br>E – 0.4 | C – 3.4<br>E – 2.2 | C – 3.9<br>E – 2.7 | C – 5.5<br>E – 3.6 |
| | | Hypocotyl | C – 0.4<br>E – 0.3 | C – 1.5<br>E – 1.1 | C – 4.1<br>E – 2.5 | C – 4.5<br>E – 3.0 |
| 5. | Hibiscus esculentus (Lady's finger) | Radical | C – 0.8<br>E – 0.5 | C – 2.3<br>E – 1.5 | C – 3.4<br>E – 2.2 | C – 4.3<br>E – 2.8 |
| | | Hypocotyl | C – 0.5<br>E – 0.4 | C – 1.4<br>E – 0.9 | C – 2.6<br>E – 1.7 | C – 3.7<br>E – 2.2 |
| 6. | Phaseolus mungo (Moong bean) | Radical | C – 0.6<br>E – 0.5 | C – 2.5<br>E – 1.8 | C – 3.5<br>E – 2.2 | C – 5.5<br>E – 3.4 |
| | | Hypocotyl | C – 0.5<br>E – 0.3 | C – 2.4<br>E – 1.5 | C – 3.1<br>E – 1.9 | C – 4.4<br>E – 2.7 |

- Length of Radical & Hypocotyl were measured in centimeters.

## Table: 2
## Effects of Guignardiacitricarpa toxins on Amylase activity in whole germinating Brassica seedlings

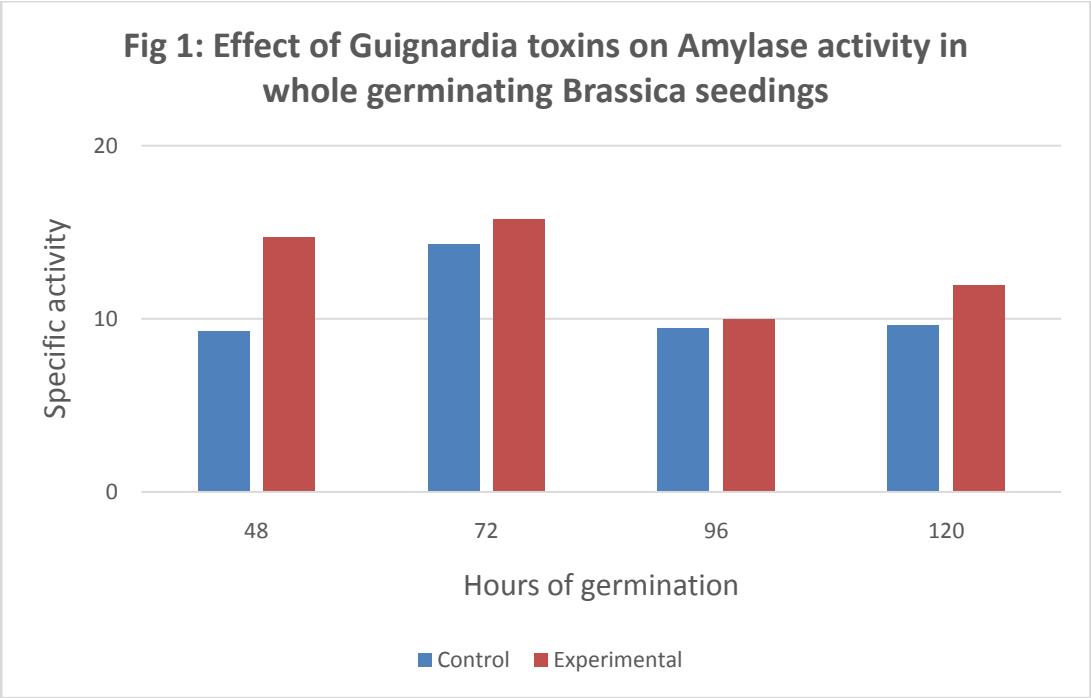
| Hours of germination | Control | | Experimental | |
|---|---|---|---|---|
| | U/g of seeds | Specific activity | U/g of seeds | Specific activity |
| **48** | 250.0 | **9.29 ± 0.164** | 394.4 | **14.71 ± 0.302*** |
| **72** | 322.2 | **14.32 ± 0.411** | 427.8 | **15.72 ± 0.289**** |
| **96** | 247.2 | **9.47 ± 0.207** | 277.8 | **9.95 ± 0.056***** |
| **120** | 191.6 | **9.62 ± 0.025** | 294.4 | **11.19 ± 0.062*** |

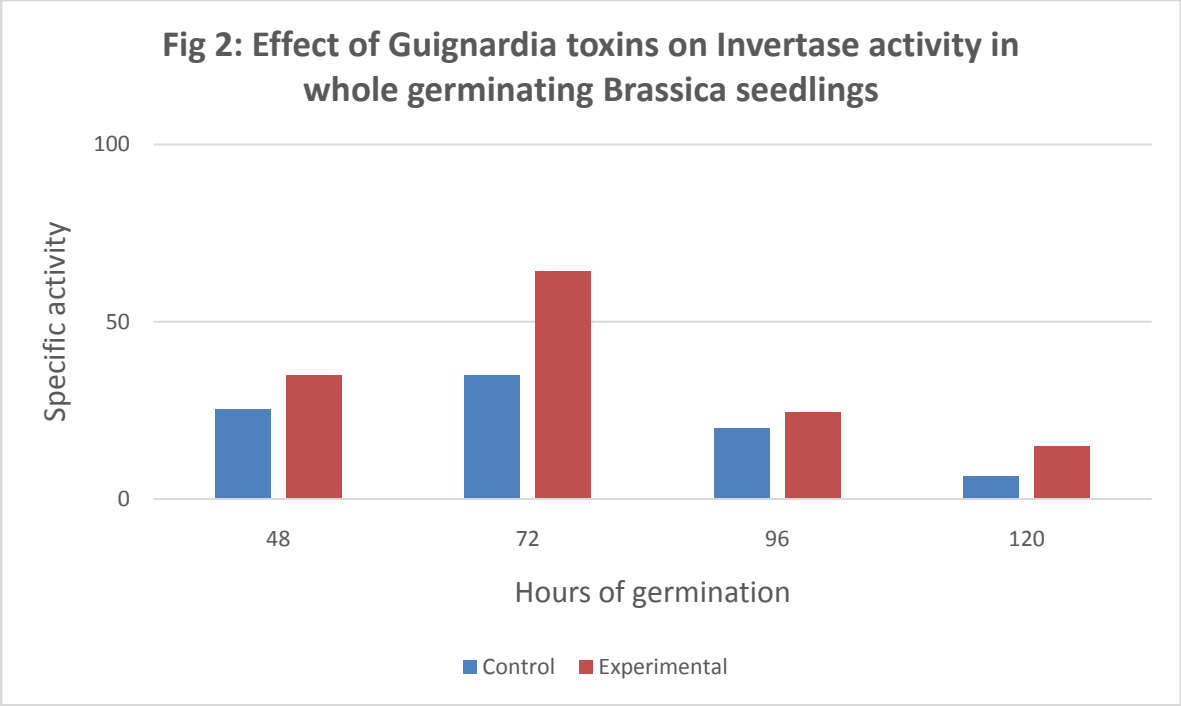
*** p < 0.001, ** p < 0.01, *** p < 0.02**

## Table: 3
## Effects of Guignardiacitricarpa toxins on Invertase activity in whole germinating Brassica seedlings

| Hours of germination | Control | | Experimental | |
|---|---|---|---|---|
| | U/g of seeds | Specific activity | U/g of seeds | Specific activity |
| **48** | 680.5 | **25.29 ± 0.144** | 1041.6 | **34.95 ± 0.204*** |
| **72** | 784.7 | **34.85 ± 0.287** | 1743.0 | **64.08 ± 0.734*** |
| **96** | 520.8 | **19.95 ± 0.238** | 680.5 | **24.39 ± 0.263*** |
| **120** | 125.0 | **6.28 ± 0. 095** | 368.0 | **14.89 ± 0.275*** |

*** p < 0.001**




## Discussion

Several workers have observed the inhibitory effect of microbial toxins on germinating seedlings. Bandre et al [10] have noticed the inhibitory effect of aflatoxin $B_1$ on the growth of radicle &

hypocotyl in **Brassica** seedlings. Similar results were observed by Schoental& White [6] in germination of seeds in **Lepidium sativum** after treatment with aflatoxin $B_1$. In the present study similar inhibitory effect of the **Guignardia** toxins on the growth of radicle & hypocotyl were noticed in germinated seedlings of **T. vulgare**, **B.juncea**, **C.tetragonoloba**, **Zea-mays**, **H. esculentus**&**P. mungo**. The effect of the toxins on germination could be due to the inhibition of cell division & cell elongation which require the release of energy & supply of the cell wall materials that are commonly derived from degradation of complex food substances.

Activities of amylase & invertase estimated in germinating **Brassica** seedlingswere affected by the **Guignardia** toxins. Though the effect on germination was inhibitous, both these enzymes have showed elevated activities in toxin treated seeds as compared to control. Similar apparent contradiction has also been reported in studies by other workers. For e.g.vanadate at low concentration has been found to stimulate seed germination but is inhibitory at higher concentration. Irrespective of whether the dose is stimulatory or inhibitory for germination, certain energy yielding enzyme activities were found to be elevated [15].

## Conclusion

In the present study, **Guignardia** toxins showed a considerable reduction in germination & growth of seedlings of **T. vulgare**, **B.juncea**, **C.tetragonoloba**, **Zea-mays**, **H.esculentus**&**P. mungo**.Remarkable increase in activities of amylase & invertase was noticed in toxin treated germinating **Brassica** seedlings.

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