

**EFFECTS OF SEED PRIMING WITH PARAMYLON  
NANOPARTICLE IN BLACKGRAM DURING ALUMINIUM STRESS  
PROJECT WORK**

Submitted in partial fulfillment of the requirements for the award of Bachelor of  
Science degree in Biotechnology

**By  
SOWMIYA. R (41770033)**



**SCHOOL OF BIO AND CHEMICAL ENGINEERING  
DEPARTMENT OF BIOTECHNOLOGY**

**SATHYABAMA**

**INSTITUTE OF SCIENCE AND TECHNOLOGY  
(DEEMED TO BE UNIVERSITY)  
CATEGORY-1 UNIVERSITY BY UGC**

**Accredited with Grade “A++” by NAAC | 12B Status by UGC | Approved by AICTE**

**JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119**

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**BONAFIDE CERTIFICATE**

This is to certify that this Project Report is the bonafide work of SOWMIYA. R (41770033) who carried out the project entitled "EFFECTS OF SEED PRIMING WITH PARAMYLON NANOPARTICLE IN BLACKGRAM DURING ALUMINIUM STRESS" under my supervision from September 2022 to April 2023.

Internal Guide

DR. INDUMATHI S M

External Guide

DR. SRINIVASAN R

Head of the Department

DR. V. RAMESH KUMAR

Submitted for Viva-voce Examination held on 27.04.2024

INTERNAL EXAMINER

(Dr. S. JAYASHREE)

EXTERNAL EXAMINER

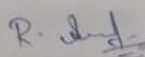
Dr. Ponbharan Sibi

## DECLARATION

I SOWMIYA.R(41770033) here by declare that the Project Report entitled "EFFECTS OF SEED PRIMING WITH PARAMYLON NANOPARTICLE IN BLACKGRAM DURING ALUMINIUM STRESS" done by me under the guidance of Dr. SRINIVASAN R, Assistant Professor (Research), Centre for Ocean Research, COL. Dr. JEPPIAR RESEARCH PARK, SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY (External) and Dr. INDUMATHI S M, Assistant Professor, Department of Biotechnology (Internal) is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biotechnology.

DATE: 27.04.24

PLACE: CHENNAI

  
THE SIGNATURE OF THE CANDIDATE

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## ABSTRACT

This study investigates the effects of seed priming with paramylon nanoparticle in blackgram during aluminum stress. Aluminium stress refers to the detrimental effects caused by excessive aluminium (Al) concentrations in the soil on plant growth and productivity. Research on aluminium stress in seeds often focuses on understanding the genetic and physiological mechanisms underlying aluminium tolerance. Paramylon, a  $\beta$ -1,3-glucan derived from the unicellular algae *Euglena gracilis*, which is rich in a unique polysaccharide called paramylon. In this research, *Vigna mungo* seeds were subjected to 100 $\mu$ g of Al stress concentration in the presence and absence of paramylon nanoparticles. Germination rate, seedling growth parameters, and biochemical markers of stress were evaluated to assess the efficacy of paramylon nanoparticles alleviating aluminium-induced inhibition of germination. Preliminary results indicate that the paramylon nanoparticles positively influence seed germination and seedling vigor under aluminium stress conditions. It is found that the highest germination rate is found in 5 $\mu$ g and 50 $\mu$ g paramylon nanoparticle concentration. Also, the ROS accumulation and ROS aluminium in the root tips of the seedlings are visualized by staining the tips in Dichlorodihydrofluorescein diacetate dye and morin dye respectively. Decreased ROS accumulation is observed in high concentration of paramylon nanoparticle coating. The higher level of anti-oxidant enzymes such as CAT, ATX and SOD are higher in the seeds that are primed with paramylon nanoparticle. These findings suggest the potential of paramylon nanoparticles as a novel approach to enhance crop resilience to aluminium stress and contribute to the development sustainable agricultural practices. Therefore, from overall study, we can understand that the seed priming of paramylon nanoparticle in blackgram seedling can effectively overcome the applied Aluminium stress condition and positively result in the overall growth characteristics of the blackgram (*vigna mungo*) seedlings.

**Keywords:** Seed germination, Nano-particles, Toxicity, Growth characteristics, Dichloro Dihydrofluorescein Diacetate, Stress condition.

## **LIST OF ABBREVIATIONS**

AL - Aluminium

BG - Black Gram

CACL<sub>2</sub> - Calcium Chloride

CC - Chlorophyll Content

DCDFA - Dichloro Dihydrofluorescein Diacetate

DW - Dry Weight

FW - Fresh Weight

SL - Shoot Length

RL - Root Length

KNO<sub>3</sub> - Potassium Nitrate

NP - Nano-Particles

PEG - Polyethylene Glycol

ROS - Reactive Oxygen Species

TPP - Triphenylphosphine

ML - Milliiter

RPM - Revolution Per Minute

GP - Germination Percentage

SVI - Seedling Vigor Index

NaOH - Sodium Hydroxide

SP - See Priming

CAT - Catalase

SOD - Superoxide

APX - Ascorbate Peroxidase

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# CHAPTER-1

## INTRODUCTION

### 1.1 BLACKGRAM

Blackgram (*Vigna mungo*), also known as urad bean, is a vital pulse crop renowned for its high nutritional value and adaptability to diverse agro-climatic conditions. It is one of the most important grain legume, plays an important role in tropical and sub-tropical of short duration legume. Blackgram is rich in dietary protein and micronutrients and it is root commonly cultivated in the area of Southeast Asia and Bangladesh (*Alsaedi et al. 2019*)

### 1.2 ALUMINIUM STRESS

Aluminium stress refers to the detrimental effects caused by excessive aluminium (Al) concentrations in the soil on plant growth and productivity. Aluminium is the most abundant metal in the earth's crust and can become toxic to plants when present in concentrations, particularly in acidic soils. Aluminium (Al) stress is a pervasive challenge confronting plants in many agricultural ecosystems, significantly impairing growth, development, ultimately, crop yields. Aluminium toxicity occurs predominantly in acidic soils, where solubilized aluminium ions hinder root growth, nutrient uptake, and physiological processes essential for plant vitality. Different seeds exhibit varying levels of tolerance to aluminium stress (*Bambang Suwinbnyo et al.,(2023)*). Some plants have mechanisms to detoxify or exclude aluminium from their roots, while others are more sensitive to its effects. Research on aluminium stress in seeds often focuses on understanding the genetic and physiological mechanisms underlying aluminium tolerance. Understanding the mechanisms by which aluminium affects plants and developing strategies to mitigate its impact are essential for sustainable crop production in affected area (*Mehrzaad Zargarzadesh et al.,2020*).

### 1.3 SEED PRIMING

One such technique gaining prominence is seed priming, a pre-sowing seed treatment method that enhances germination, seedling vigor, and overall crop performance. Seed priming involves controlled hydration and dehydration of seeds

before planting, triggering physiological and biochemical changes that confer resilience to environmental stresses (*F Zhu et al., 2016*). This process stimulates early seedling growth, leading to improved crop establishment, uniform emergence, and ultimately, higher yields. Common priming solutions include water, potassium nitrate ( $\text{KNO}_3$ ), calcium chloride ( $\text{CaCl}_2$ ), and polyethylene glycol (PEG) (*Feizi et al., 2012*). In this case of black gram, seed priming holds immense potential to mitigate challenges associated with sub optimal soil conditions, erratic rainfall patterns, and pest pressures, thereby bolstering productivity and farmer livelihoods. In this primer, we delve into the rationale behind seed priming in black gram cultivation, exploring its mechanisms, benefits, and practical application strategies (*Mehrzaad Zargarzadesh et al., 2020*).

#### 1.4 PARAMYLON FROM *EUGLENA GRACILIS*

Paramylon, a  $\beta$ -1,3-glucan polysaccharide derived from the unicellular algae *Euglena gracilis*, which is rich in a unique polysaccharide called paramylon. These are emerged as a promising candidate in the realm of nanotechnology, particularly in the form of paramylon nanoparticles. These nanoparticles, with their unique physicochemical properties and biocompatibility, hold tremendous potential for diverse applications in biomedicine, ranging from drug delivery systems to immunomodulatory agents (*Sahandi et al., 2011*).

#### 1.5 THE MECHANISM OF NP'S UPTAKE IN PLANT ROOTS

The mechanism of NPs uptake by plant roots is not clearly understood. Studies have shown that depending on the size, NPs may enter in plant cell through carrier proteins, ion channels, via fluid phase endocytosis, plasmodesmata transport, or entry may be facilitated through natural organic matter or root exudates and formation of new pores (*Singh et al., 2021*). The uptake and transport of NPs are size dependent, and they move actively as well as passively throughout the plant. Furthermore, it was discovered that once NPs enter different tissues, they are effective at enhancing specific morphophysiological parameters like height and flower count, fruit yield fresh and dry weights, overall growth and biomass, early flowering, total crop yield, quantity and quality in soybean and mung bean, seed germination, and uptake of macro and micronutrients (*Kole et al., 2013*).

Thus, newer developments in the agricultural sector are mandatory such as applying new technologies and continuous innovation in agriculture is highly needed. Nanotechnology is one of the most promising and innovative tools in the current scenario and has been acknowledged as “Key Enabling Technology” by the European Commission (*N. Singh, Bhuker, and Jeevanadam et al., 2021*).

#### 1.6 SEED PRIMING IN BLACKGRAM

Seed priming is a pre-sowing treatment where seeds are soaked in water or a solution to initiate the metabolic processes necessary for germination. In blackgram, seed priming can enhance germination rate, uniformity, and seedling vigor, ultimately leading to better crop establishment and yield. The process typically involves soaking seeds in water or a nutrient solution for a specific period, followed by drying before sowing (*Ali et al., 2019*).

Seedlings were randomly selected for each treatment to observe seedling parameters, such as shoot length and root length, fresh weight and dry weight of the seedlings. Shoot length and root length were measured using centimetre scale. For estimation of dry weight, seedlings were dried in hot air oven at 80°C for 24 hours and expressed in gram .

## CHAPTER-2

### LITERATURE SURVEY

Bioefficacy of Fe<sub>2</sub>O<sub>3</sub> quantum dots on enhancing seed germination and seedling vigour in blackgram (*Vigna mungo*) QDs are capable of entering the seeds through pores present on the seed coat during water imbibition, induce water uptake ability of seeds, and enhance enzymatic activity. The present study demonstrates that application of Fe<sub>2</sub>O<sub>3</sub> Quantum dots to improve Black gram seed quality. At the concentration of 500 ppm, Fe<sub>2</sub>O<sub>3</sub> Quantum dots improve the seed germination, seedling length, seedling dry weight and seedling vigour. When the concentration of QD increases beyond 500 ppm, it shows antagonistic effect on the seed parameters (*P et al.,2022*)

Biogenic ZnO and Cu nanoparticles to improve seed germination quality in blackgram (*Vigna mungo*) ZnO and Cu nanoparticles have significant impact in improving seed quality of black-gram (*Vigna mungo*). Seeds of *vigna mungo* were soaked with different concentration of nanoparticles. And results were observed after 2-3 months (*Raja et al.,2018*)

Copper nanoparticle application enhances plant growth and grain yield in maize under drought stress conditions. Copper oxide NPS positively affects plant biomass under both normal and drought conditions. Additionally, application of Copper oxide NPS retains leaf water status, as well as chlorophyll and carotenoid content under drought stress conditions. Copper oxide NPS priming has a major role in controlling ROS level in maize in response to water stress through increasing both ROS-scavenging enzyme activity and antioxidants (*Van Nguyen et al.,2022*)

Effects of copper nanoparticles (CuO NPs) on crop plants: a mini review. 14 The toxicity of NPs depends on plant species, growth conditions, exposure time, concentration, type, and size of NPs. It is well noted that the Cu ions in small concentrations are able to stimulate the plant growth and play the role as microelement (*Rajput et al.,2018*)

Effects of metal nanoparticle-mediated treatment on seed quality parameters of different crops It is concluded that the toxicity of the nanoparticles toward the environment as well as crops depends on the type, chemical composition, size,

density, method of preparation, and application of nanoparticles. Further, the type and age of the crop as well as the duration of treatment of a specific nanoparticle toward a particular crop can also play a crucial role in their toxicity (*N. Singh et al., 2021*)

Effects of Nanoparticles on Seed Germination, Growth, Phytotoxicity and Crop Improvement. Various types of metallic NPs released in our natural environment have a great impact on plant species. Therefore, nanotechnology is becoming very essential today to further help promote ultra-modern farming systems with low environmental impact. Synthesized NPs were used in a wide variety of applications for crop growth and higher yield. It regenerates and accumulates in plant roots, stems, leaves and other parts of the plant and promotes plant growth, respiration, transpiration, biomass production, yield improvement and phytotoxicity. NPs have produced both positive and negative effects on plant growth and development, which depends on the size and shape of NPs (*Verma et al., 2020*)

Green synthesis, characterization and applications of nanoparticles. Synthesized metal oxide nanoparticles were characterized using UV spectroscopy, SEM, FTIR and XRD. The peak range observed for zinc oxide nanoparticles ranges between 350-380nm and for copper oxide and iron oxide nanoparticles the observed range between 220-280nm (*Pal et al., 2019*)

Impact of green synthesized metal oxide nanoparticles on seed germination and seedling growth of *Vigna radiate* (*Mung Bean*) and *Cajanus cajan* (Red Gram). Similar to this paper, comparative analysis can also be made among copper oxide, iron oxide and zinc oxide nanoparticles, observing which nanoparticle has shown better results considering different parameters such as root and shoot length, biomass, antioxidant activity (*Rani, P et al., 2020*)

Impact of nanoparticles on abiotic stress responses in plants: an overview It is essential to gain basic knowledge about the interaction of NPs with plants at various levels to exploit the best properties of NPs. NPs show their impact at very low concentrations, and the effect on plants is type and dose dependent. The scientific community has a major concern to overcome abiotic-stress-induced loss of crop productivity, and several NPs are being studied to assess their potential role

in protecting plants from abiotic stresses, plant development, crop production, and modulating the various plant processes. Additionally, to prevent adverse effects of NPs on the environment, proper understanding of their role is essential: to reduce negative impacts of NPs such as ZnO NPs on the environment and crops, it is vital to engineer their properties and understand plant NP interactions better before their practical application in the field (*Z. Khan et al., 2019*)

**Seed priming with nanoparticles:** An emerging technique for improving plant growth, development, and abiotic stress tolerance. The excess usage of chemical stimulants is responsible for the reduction of crop yield and destruction of soil fertility and the ecosystem. Seed priming with NPs is a promising tool that can be employed to promote successful crop production. The literature showed that the use of NPs-priming has the latent to change the conventional agriculture based on the practice of agro-chemicals into a more maintainable agriculture, as these systems can improve plant growth and mitigate abiotic stressors, consequently, ensuring in increments of productivity and quality of foods (*Rhaman et al., 2022*)

The use of bio-stimulants or osmoprotectants has been one of the most practical approaches so far to overcome environmental and abiotic stress. Not only stressed plants but also their transgenic expression would be beneficial with the use of osmoprotectant such as proline, glycine betaine, or amino acids. In this chapter, under abiotic stress conditions as well as their significance and expressions under biotic stress conditions, the role of osmoprotectants has been evaluated (*Murat Dikilitas et al., 2020*)

The use of nanoparticles and Nano formulations in agriculture. 13 Nanoparticles have great potential to improve the agricultural productivity. Toxicity for humans must also be taken into account because in many cases the nanoparticles cannot be fully removed from human-consumed agricultural products (*Pestovsky et al., 2019*)

TiO<sub>2</sub> nanoparticles can enhance germination and seedling growth of mung bean (*Vigna radiata* L.). The uptake rate depends on the size and the surface properties of the nanoparticles. Nanoparticles could enter the xylem via the cortex and the central cylinder and may accumulate in the vacuole. The increase in seed

germination along with seedling growth in specific concentration of nanoparticles suggested optimum dose limit on growth of seedlings (*Priya et al.,2020*)

To examine the ZhongmuNo3 alfalfa seed germination stage with 200mM NaCl and 180g.L-1 Polyethylene glycol (PEG) physiological and proteomic analyses were applied. Through two-dimensional electrophoresis (2-DE), more than 800 proteins were detected by proteomic technology. Discovered that under PEG-mediated drought stress and salt stress, some proteins related to carbohydrate, metabolism and energy production increased in abundance. Overall this study demonstrated a common mechanism of energy consumption during abiotic stress (*Qiaoli Ma et al.,2017*)

## **CHAPTRE-3**

### **AIM AND OBJECTIVES**

#### **3.1 AIM**

To determine the effects of seed priming with paramylon nano-particles in blackgram during aluminium stress.

#### **3.2 OBJECTIVES**

- Priming the *Vigna mungo* seeds with  $\beta$ -1,3 glucan nanoparticle of different concentration.
- Introducing the primed seeds to the stress condition (Aluminium stress) for germination.
- Analyzing the germination rate, seedling growth and other characteristics of the primed seeds in comparison with unprimed seeds (control).
- Overview about the effect of paramylon nanoparticle in overcoming the applied stress condition.

## CHAPTER-4

### MATERIALS AND METHODS

#### 4.1 MATERIALS REQUIRED

The materials obtained in this experiments are Blackgram, Paramylon from *Euglena gracilis* 100mg (0.1%), 40ml Beaker, 15ml falcon tubes, Magnetic stirrer, Water (H<sub>2</sub>O) (50 ML), Sodium hydroxide crystals (0.5g), Triphenylphosphine (TPP) (4.17ml), Acetic acid 250μl (1%), Centrifuge, Ethanol, Aluminium stress (100μg), Stock solution (0.1M), Water – 10ml, 4 different concentration of Paramylon, Sterilized alfalfa seeds, Sterile water, 15 ml falcon tubes, Germination paper, Distilled water, 100ml beaker, Primed seeds, Forceps, Rubber bands & tissue paper, DCFDA (2'-Dichlorodihydrofluorescein diacetate), Buffer, Root tips, 0.1 gm leaves, 5.0 ml of leaves and 0.1 Potassium phosphate buffer (pH 7.0).

#### 4.2 METHODS

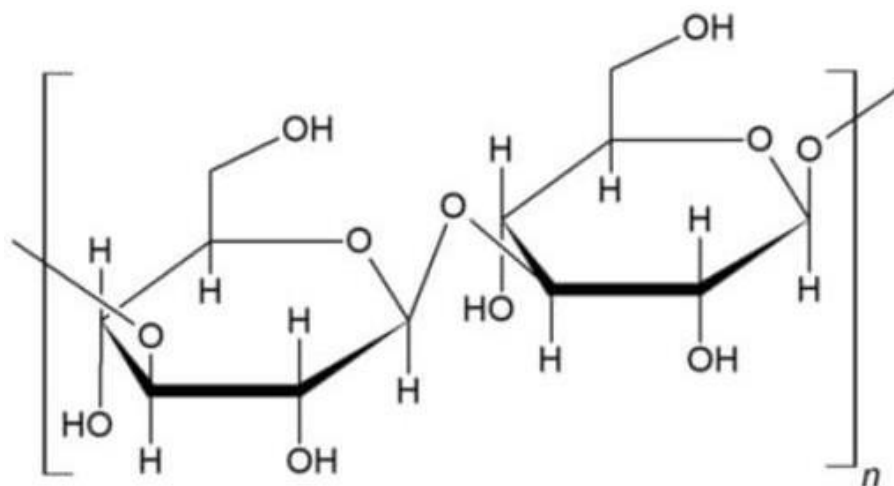
##### 4.2.1 Seed collection



**Fig 4.1 Blackgram**

Here sample refers to blackgram [*Vigna mungo*] seeds precured from sakshi farm which are non-genetically modified organic (GMO) and chemical free and 100% organic in nature.

#### 4.2.2 Preparation of nanoparticle



**Fig 4.2 Structure of Paramylon nanoparticle**

We use the paramylon extracted from the algae *Euglena gracilis*. In 40ml of beaker, 25ml of H<sub>2</sub>O is mixed with 2% (0.5g) sodium hydroxide (NaOH). 100mg (0.1%)  $\beta$ -glucan dissolved in 25ml of 2% (0.5g) sodium hydroxide. Then kept for 2 hours in magnetic stirrer at 90°C with 420 rpm. After 2 hours of incubation, precipitated by using 1% acetic acid (250 $\mu$ l). To obtain the precipitate, the solution is kept in magnetic stirrer for 1 hour, after adding acetic acid. Fill the solution equally to 2 falcon tubes of 15ml. Centrifuge the solution at 7000 revolutions per minute (rpm) for 15 minutes at 11°C, is crucial for isolating and concentrating the nano-particles from the solution.

After centrifuge, a pellet is obtained from the solution. Triphenylphosphine (TPP) is prepared at a concentration of 2mg/ml for the experiment. The pellet was dissolved in 8.33ml of solution by adding 4.16ml of sterile water followed by 4.17ml of TPP dropwise. After being isolated for 30 minutes, the mixture is centrifuged again at 7000 rpm for 15 minutes to separate any remaining particles or impurities. The obtained pellet is washed several times and then stored in cold conditions for 24 hours to maintain its stability. The final concentration of a Nano-particle is 4mg/ml.

#### **4.2.3 Seed priming**

Before priming, seeds are sterilized by immersing them in a 2% sodium hydroxide for 10 minutes to ensure cleanliness and prevent microbial contamination. Seed priming in blackgram is involved soaking the seeds in paramylon solution for 24 hours to enhance germination and early seedling vigor. Here seed priming is conducted using four distinct concentration of paramylon nano-particle.

The four distinct concentrations are 5 $\mu$ g, 25 $\mu$ g, 50 $\mu$ g, 100 $\mu$ g. After the completion of seed priming, the seeds are for the next process. Germination, which will initiate their growth into plants.

#### **4.2.4. Nacl solution preparation**



**Fig 4.3 Aluminium Stress**

The selected aluminium concentration was 100 $\mu$ m. The aluminium solution was prepared by dissolving 8.766g of all in 10ml of water. From the Al stock solution, 250 $\mu$ l of Al stress is combined with 250 $\mu$ l of water creating a solution that as then used for germination.

#### 4.2.5 Incubation of seeds for germination



**Fig 4.4 Control seedlings of Blackgram  
without PNP treatment**



**Fig 4.5 Blackgram seed priming with  
NPN under Aluminium stress**

After 24 hours of priming, the seeds are now prepared and ready to be placed for germination. For the germination process, a total of six rolls of germination paper have been prepared, encompassing a control group, stress conditions, and for distinct concentration on nano-particle. In the control group, 8 unprimed blackgram seeds are positional within germination paper to facilitate germination, then bundled with a rubber band and positioned in a beaker containing only 250µl of water. In a beaker containing 250µl of distilled water combined with 100µg of Al stress as mentioned in working standard, 8 unprimed blackgram seeds are positional within germination paper to facilitate germination then bundled with a rubber band to avoid opening of germination paper. For aluminium (Al) stress 4.7 PH is maintained.

The primed seeds (8 seeds) with a concentration of 5µg of paramylon nanoparticles are positioned within germination paper to facilitate germination are placed in the beaker containing 250µl of water combined with 100µg of Al solution. The primed seeds (8 seeds) with a concentration of 25µg of paramylon nanoparticles are positioned within germination paper to facilitate germination are placed in the beaker containing 250µl of water combined with 100µg of Al stress. The primed seeds (8 seeds) with a concentration of 50µg of paramylon nanoparticles are positioned within germination paper to facilitate germination are placed in the beaker containing 250µl of water combined with 100µg of Al strss. The primed seeds (8 seeds) with a concentration of 100µg of paramylon nanoparticles are positioned within germination paper to facilitate germination are placed in the beaker containing 250µl of water combined with 100µg of Al stress.

These seeds are kept for germination for 7days at a temperature range of 27°C – 30°C. The water and stress levels in the beaker, where the seeds are positioned are changed at intervals of 2days to ensure there was no contamination and to maintain a consistent stress concentration. After 7 days of germination, further analysis including germination rate, root length and shoot length dry weight and fresh weight and seedling vigor are conducted.



***Fig 4.6 Germination process of Blackgram seedling***



***Fig 4.7 Blackgram seedling after germination***

#### 4.2.6 ROS accumulation

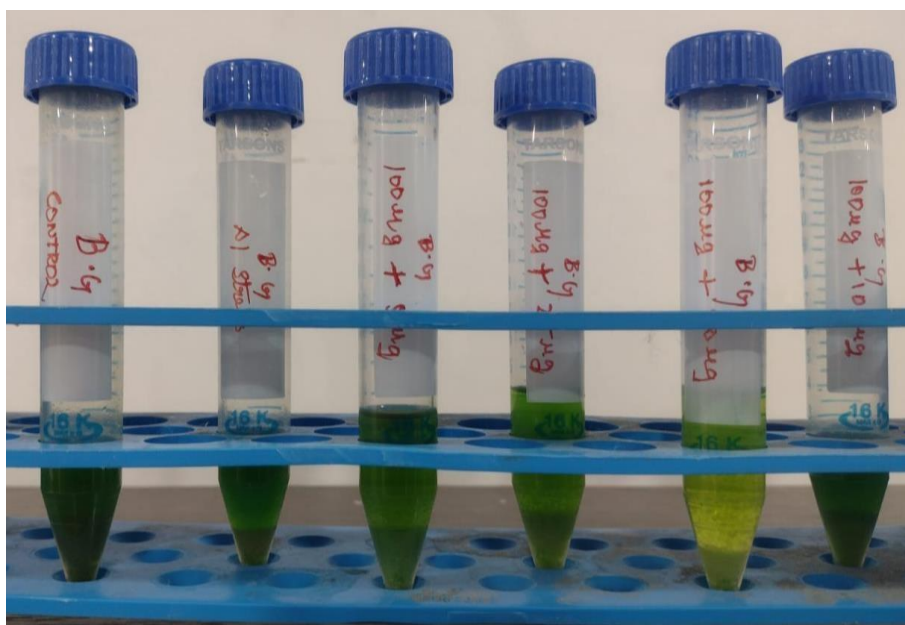


**Fig 4.8 Root tips from Blackgram seedling for ROS accumulation**

- To stain root tips for ROS (Reactive Oxygen Species) accumulation using DCFDA (2' – Dichlorodihydrofluorescein diacetate).

Dilute DCFDA stock solution in a suitable buffer or medium. Typically, a final concentration of 10-50  $\mu\text{M}$  is used. Harvest root tips to Excise the root tips from the plants of blackgram. Ensure they are intact and healthy. Incubate with DCFDA solution then place the root tips in a small container or well plate and cover them with the prepared DCFDA working solution. Incubate them in the dark for about 30 minutes to allow the DCFDA to penetrate the cells and be cleaved by intracellular esterase, resulting in the formation of the fluorescent compound DCFDA (2' – Dichlorodihydrofluorescein diacetate). After the incubation period, wash the root tips with a buffer or medium to remove excess DCFDA. Mount the tips on a microscope slide and observe them under a fluorescence microscope equipped with appropriate filters for detecting DCFDA fluorescence emitted by DCFDA. Quantify the fluorescence intensity using image analysis software to compare ROS levels among different samples or conditions.

#### 4.2.7 Chlorophyll Content



**Fig 4.9 Chlorophyll extraction from Blackgram seedlings**

Chlorophyll content was estimated following the protocol briefly, 0.1gm leaves were homogenized in 5.0 ml of acetone and centrifuged at 4000 rpm for 20 minutes. The supernatant was collected, and the residue was re-extracted twice with 5ml of acetone and centrifuged. The supernatants were combined and volume was made to 20 ml with 80% acetone.

#### 4.2.8. Antioxidant assay

The leaves of blackgram seedlings were homogenized on ice using a motor and pestle in a 0.1M potassium phosphate buffer (pH 7.0), followed by the centrifugation at 12000 rpm for 15 minutes at 4°C. The resulting supernatant was utilized for enzyme assays for (CAT- Catalase Activity, SOD- Superoxide Dismutase Activity, and APX- Ascorbate Peroxidase Activity).

## CHAPTER-5

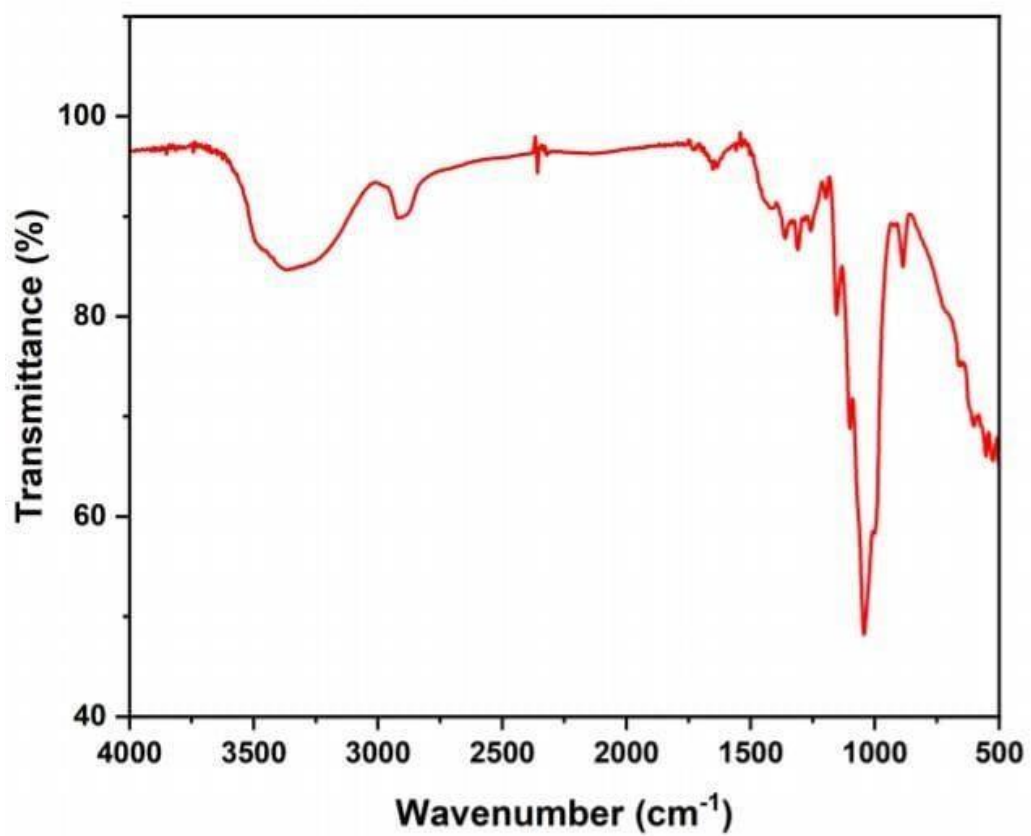
### RESULTS AND DISCUSSION

#### 5.1 FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY RESULT OF PARAMYLON NANOPARTICLE

FTIR (Fourier Transform Infrared Spectroscopy) results for paramylon nanoparticles provide valuable information about the chemical composition and functional groups present in the nanoparticles.

FTIR spectra can identify specific functional groups present in paramylon nanoparticles, such as hydroxyl (OH), carbonyl (C=O), and aliphatic groups. This information helps in understanding the molecular structure of paramylon and its potential interactions with other compounds. FTIR analysis can confirm the purity of paramylon nanoparticles by detecting the absence or presence of impurities based on characteristic absorption bands. FTIR spectra can provide insights into the molecular structure and conformational changes of paramylon nanoparticles, which is important for understanding their stability and behavior under different conditions. FTIR analysis can be used to study the interactions between paramylon nanoparticles and other molecules or surfaces, such as drugs or biomolecules, by observing changes in peak intensities or shifts in absorption bands.

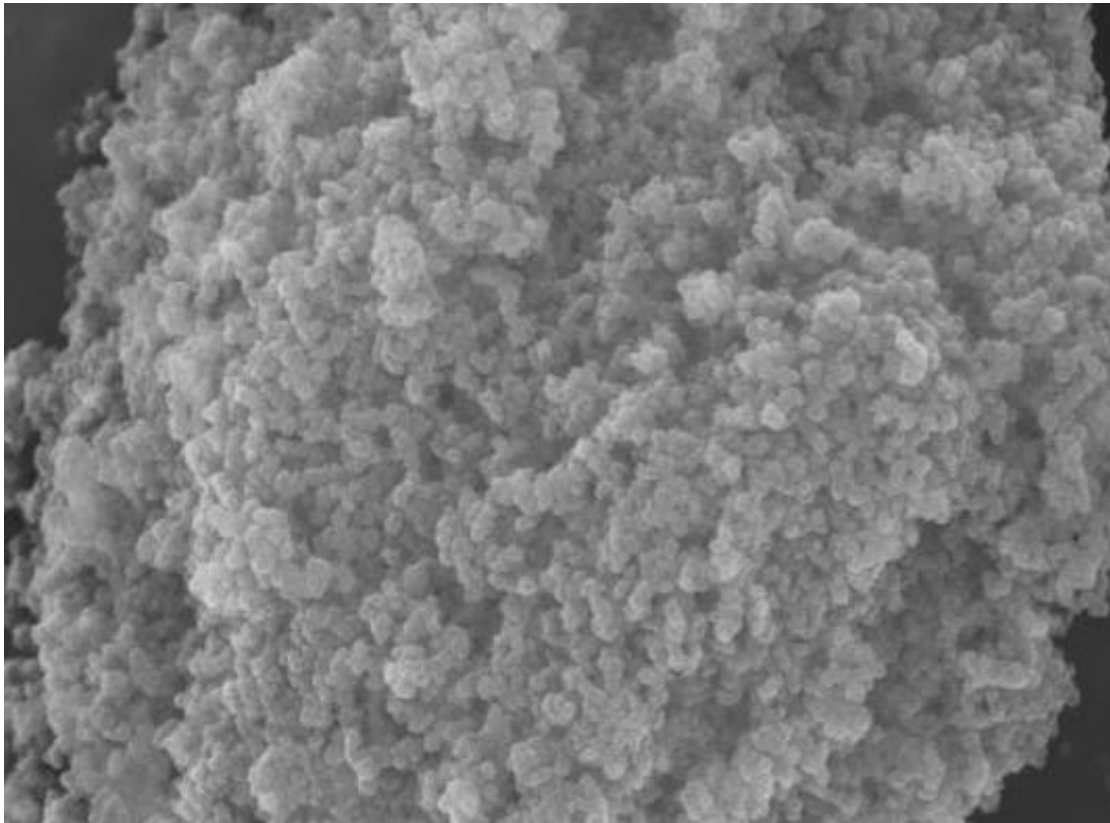
- Peak was observed at  $1020\text{cm}^{-1}$  which indicates that strong bond of C-OH.
- Vibrations near  $1480\text{cm}^{-1}$  to  $1440\text{cm}^{-1}$  indicate  $\text{CH}_2$  bond at a medium level.



*Fig 5.1 FTIR-spectrum of paramylon nano-particle*

## 5.2 SCANNING ELECTRON MICROSCOPY (SEM) RESULT OF PARAMYLON NANOPARTICLE

When applied to blackgram nano-paramylon, SEM can reveal details about the morphology, size, and distribution of the nano-paramylon particles. SEM is a powerful imaging technique used to observe the surface structure of materials at a high resolution. Additionally, SEM images can show the distribution of nanoparticles and any aggregation or clustering behavior.



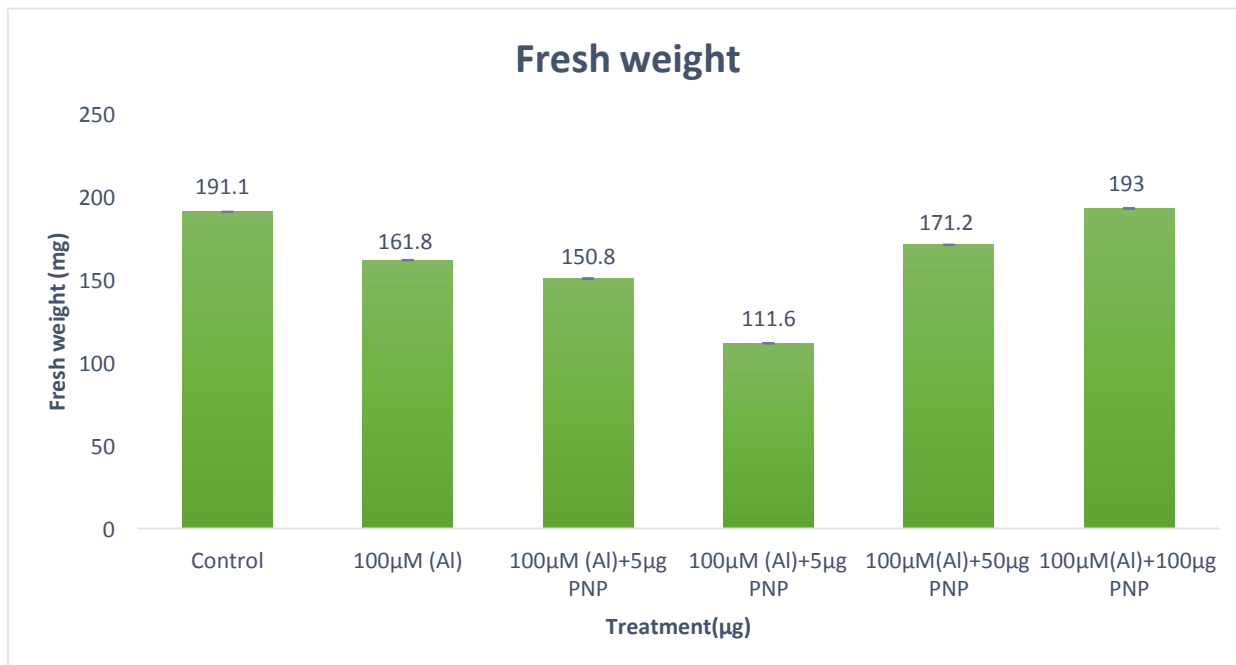
***Fig 5.2 SEM Images of paramylon nanoparticle***

### 5.3 FRESH WEIGHT

The table 5.1 represents the fresh weight of the primed blackgram seeds under aluminium stress. After 7 days of germination the plants, firstly the plants weight is observed and it is known as fresh weight. Figure 5.3 shows the graphical representation of root length and its standard deviation. Under aluminium stress conditions, the highest weight of 0.2116 was obtained in 25µg paramylon nano-particle primed blackgram seedling.

| BLACKGRAM | FRESH WEIGHT (g) |
|-----------|------------------|
| CONTROL   | 0.1911 ± 0.07    |
| Al STRESS | 0.1680 ± 0.2     |
| 5µg PNP   | 0.1508 ± 0.12    |
| 25µg PNP  | 0.2116 ± 0.16    |
| 50µg PNP  | 0.1712 ± 0.18    |
| 100µg PNP | 0.1930 ± 0.24    |

**Table 5.1 Fresh weight of Blackgram seedling treated with PNP under aluminium stress**



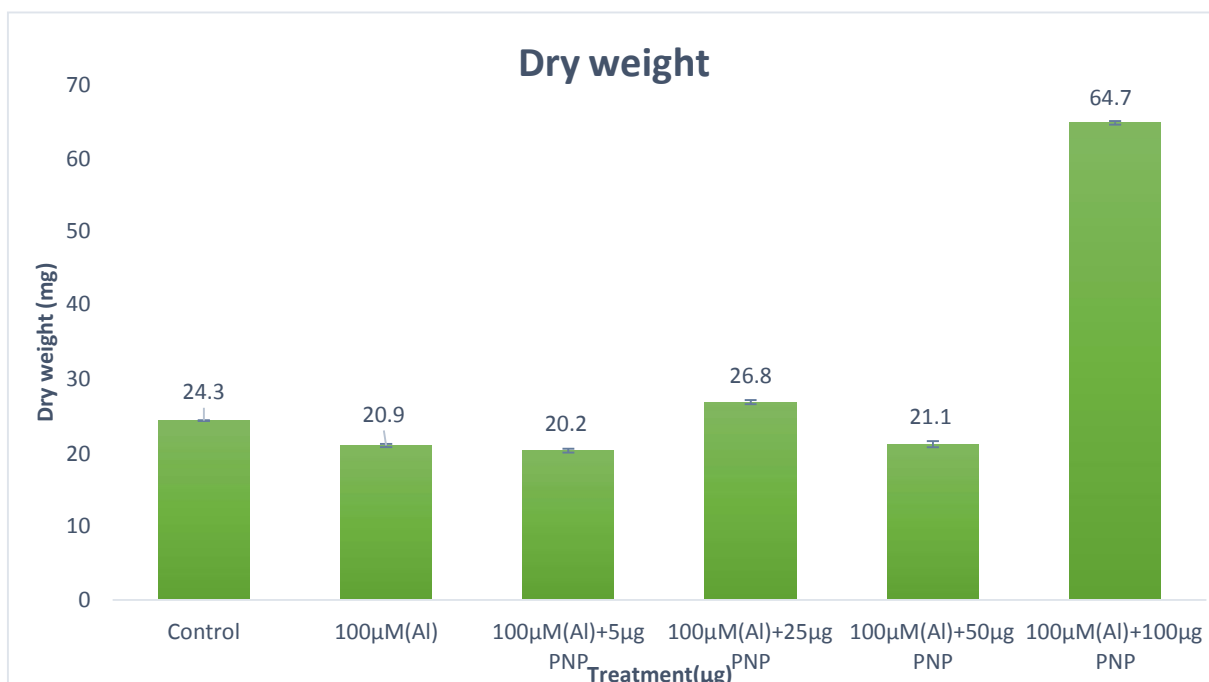
**Fig 5.3 Fresh weight of blackgram seedling treated with PNP under aluminium stress**

#### 5.4 DRY WEIGHT

The table 5.2 represents the dry weight of the primed seeds under aluminium stress. Dry weight is measured using the weighing machine in grams. The black gram plants are kept in incubator for 48 hours at 80<sup>0</sup> C to make the plants dry completely. The table 5.2 represents the dry weight of primed plants under salt stress. The highest dry weight is observed in 100µg PNP primed blackgram seedling.

| BLACKGRAM | DRY WEIGHT (g) |
|-----------|----------------|
| CONTROL   | 0.0244 ± 0.21  |
| Al STRESS | 0.0210 ± 0.25  |
| 5µg PNP   | 0.0202 ± 0.29  |
| 25µg PNP  | 0.0268 ± 0.4   |
| 50µg PNP  | 0.0211 ± 0.24  |
| 100µg PNP | 0.0216 ± 43.1  |

**Table 5.2 Dry weight of Blackgram seedling treated with PNP under aluminium stress**



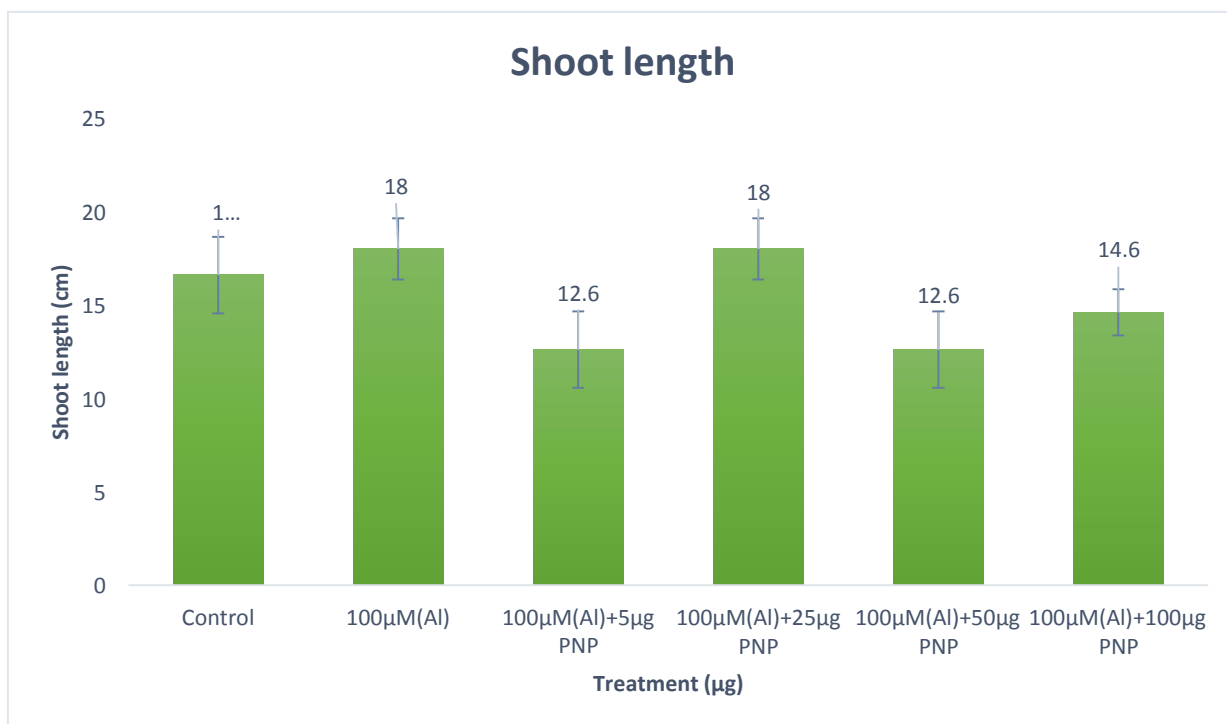
**Fig 5.4 Dry weight of blackgram seedling treated with PNP under aluminium stress**

## 5.5 SHOOT LENGTH

The table 5.3 represents the shoot length of germinated seedling, which is taken after 7 days of germination. The measuring scale is used to determine the plant's shoots length in centimetres. Figure 5.5 shows the graphical representation of standard deviation of shoot length. The highest shoot length is found in 25µg PNP primed blackgram seedling.

| BLACKGRAM | SHOOT LENGTH (cm) |
|-----------|-------------------|
| CONTROL   | 17 ± 2.05         |
| Al STRESS | 18 ± 1.63         |
| 5µg PNP   | 13 ± 2.05         |
| 25µg PNP  | 18 ± 1.63         |
| 50µg PNP  | 13 ± 2.05         |
| 100µg PNP | 15 ± 1.24         |

**Table 5.3 Shoot length of Blackgram seedling treated with PNP under aluminium stress**



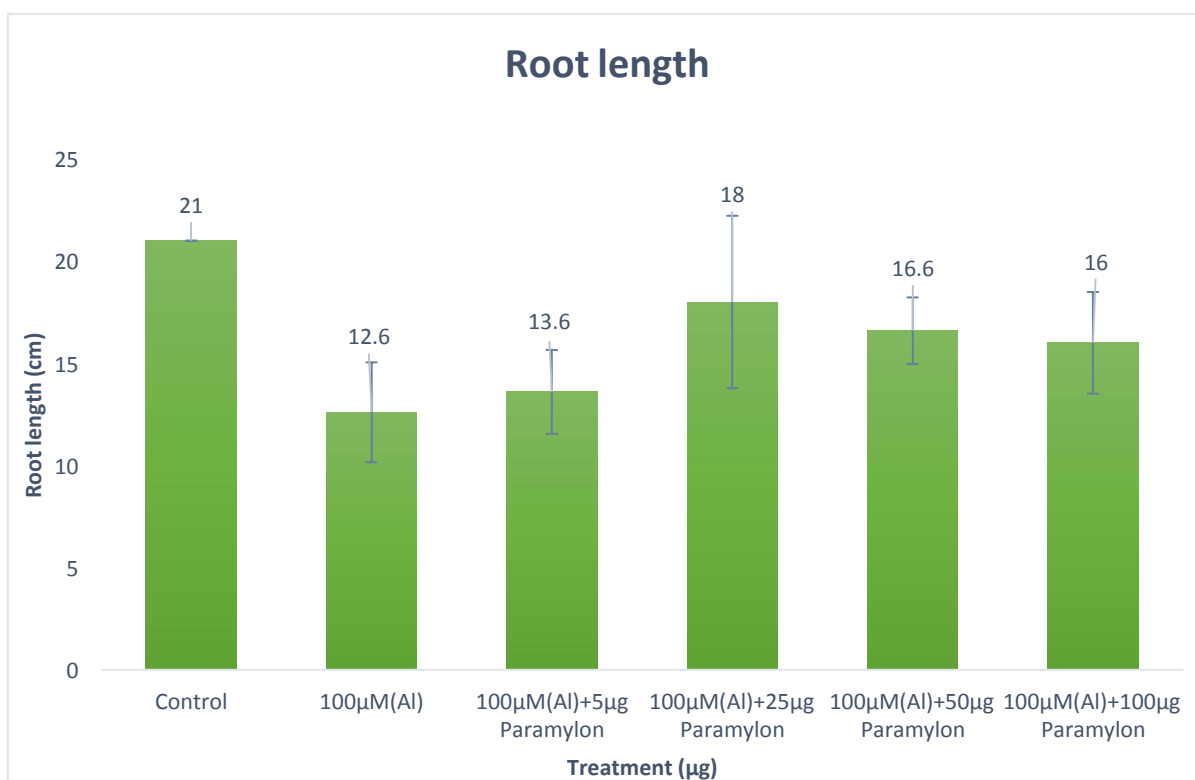
**Fig 5.5 Shoot length of blackgram seedling treated with PNP under aluminium stress**

## 5.6 ROOT LENGTH

The table 5.4 represents the root length of the germinated black gram seedling, which is taken after the seven days kept for germination. The measuring scale is used to determine the lengths of the plant's roots in centimeters. Figure 5.4 shows the graphical representation of root length and its standard deviation. After control the highest root length is achieved by 25µg PNP primed blackgram seedling.

| BLACKGRAM | ROOT LENGTH (cm) |
|-----------|------------------|
| CONTROL   | 21 ± 2.44        |
| Al STRESS | 12.6 ± 2.05      |
| 5µg PNP   | 13.6 ± 4.22      |
| 25µg PNP  | 18 ± 1.63        |
| 50µg PNP  | 16.6 ± 2.49      |
| 100µg PNP | 16 ± 1.63        |

**Table 5.4 Root length of Blackgram seedling treated with PNP under aluminium stress**



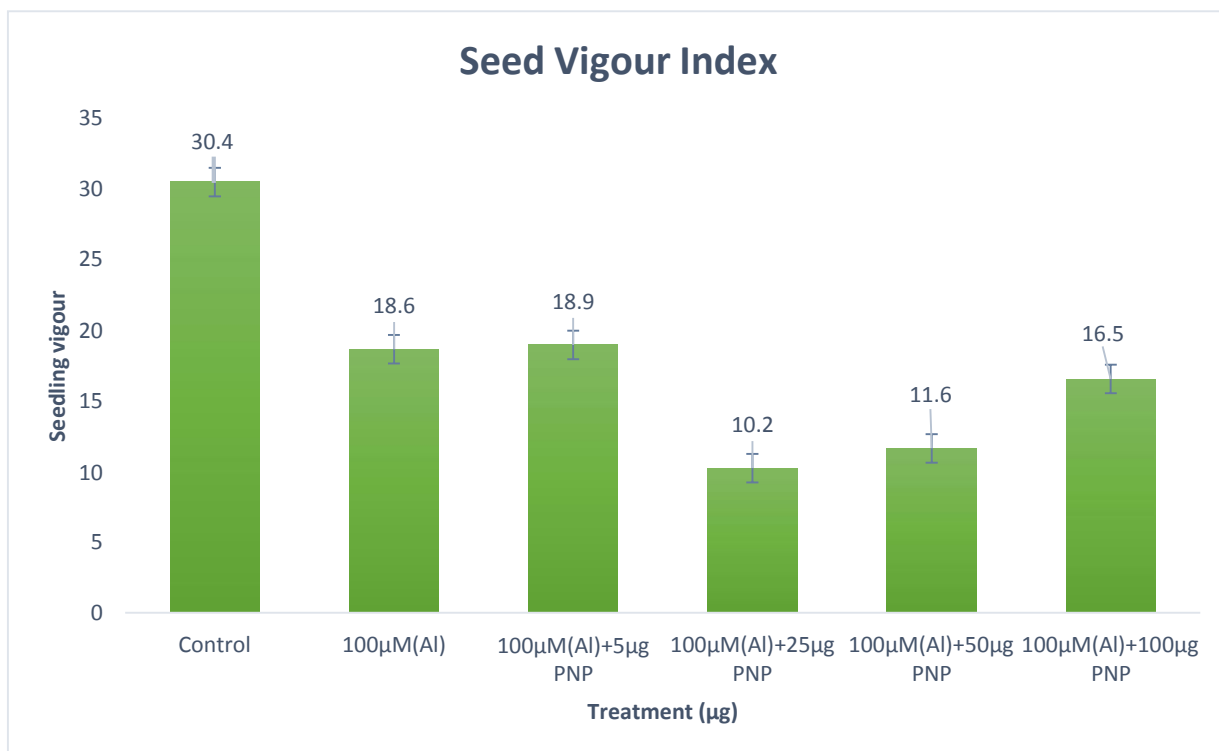
**Fig 5.6 Root length of blackgram seedling treated with PNP under aluminium stress**

## 5.7 SEEDLING VIGOR

The table represents the seedling vigor index of the germinated seeds. The vigor rate is calculated with the formula, Root length + shoot length  $\times$  germination rate  $\div$  100. The following graph represents the seed vigor of the black gram under aluminium stress. The highest seed vigor is found in the 5 $\mu$ g concentration of nanoparticle under 100 $\mu$ M aluminium stress.

| BLACKGRAM       | SEED VIGOR    |
|-----------------|---------------|
| Al STRESS       | 30.4 $\pm$ 38 |
| CONTROL         | 18.6 $\pm$ 31 |
| 5 $\mu$ g PNP   | 18.9 $\pm$ 27 |
| 25 $\mu$ g PNP  | 10.2 $\pm$ 34 |
| 50 $\mu$ g PNP  | 11.6 $\pm$ 29 |
| 100 $\mu$ g PNP | 16.5 $\pm$ 33 |

**Table 5.5 Vigor index of Blackgram seedling treated with PNP under Aluminium stress**



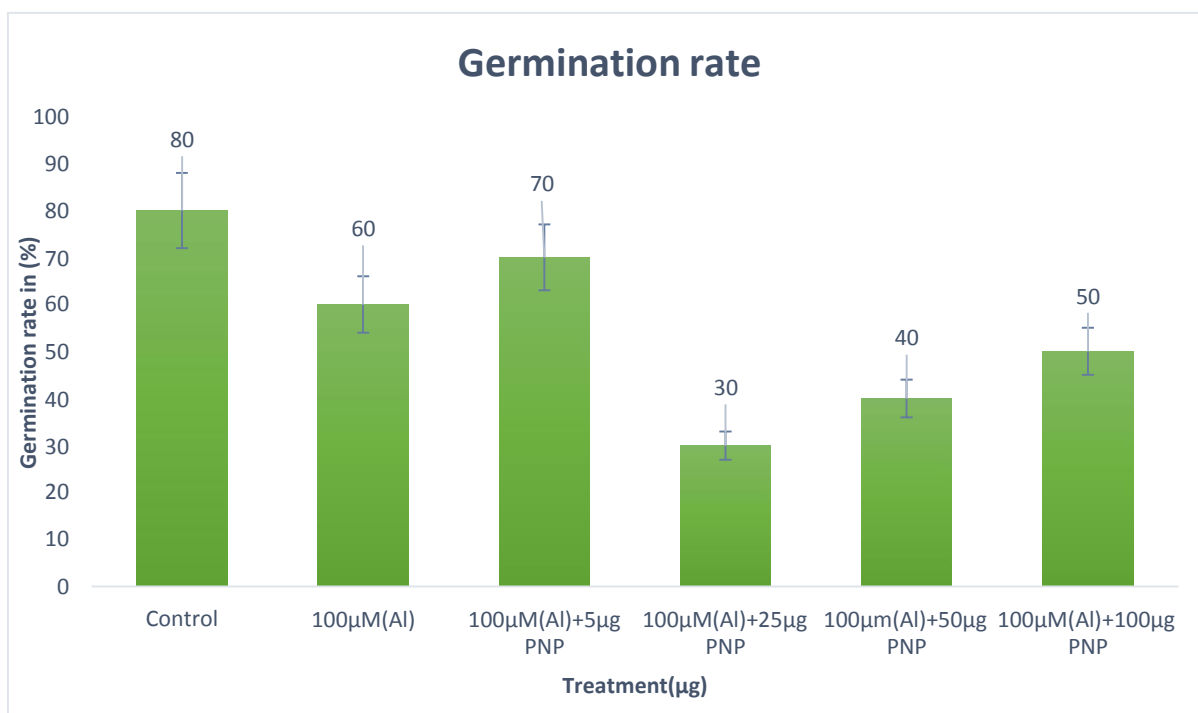
**Fig 5.7 Vigor index of Blackgram treated with PNP under Aluminium stress**

## 5.8 GERMINATION RATE

The table 5.6 represents the germination rate of *vigna mungo* plant under 100µm aluminium stress. Germination rate of seeds are observed in the intervals of 2 days and noted. 70% is the highest germination rate observed in 5µg concentration of *paramylon* nanoparticle under aluminium stress. Germination rate is calculated with the formula, No. of. Seeds germinated ÷ Total no. of seeds per plate × 100.

| BLACKGRAM | GERMINATION RATE (%) |
|-----------|----------------------|
| CONTROL   | 80 ± 8               |
| Al STRESS | 60 ± 6               |
| 5µg PNP   | 70 ± 7               |
| 25µg PNP  | 30 ± 3               |
| 50µg PNP  | 40 ± 4               |
| 100µg PNP | 50 ± 5               |

**Table 5.6 Germination rate of Blackgram seedling treated with PNP under Aluminium stress**

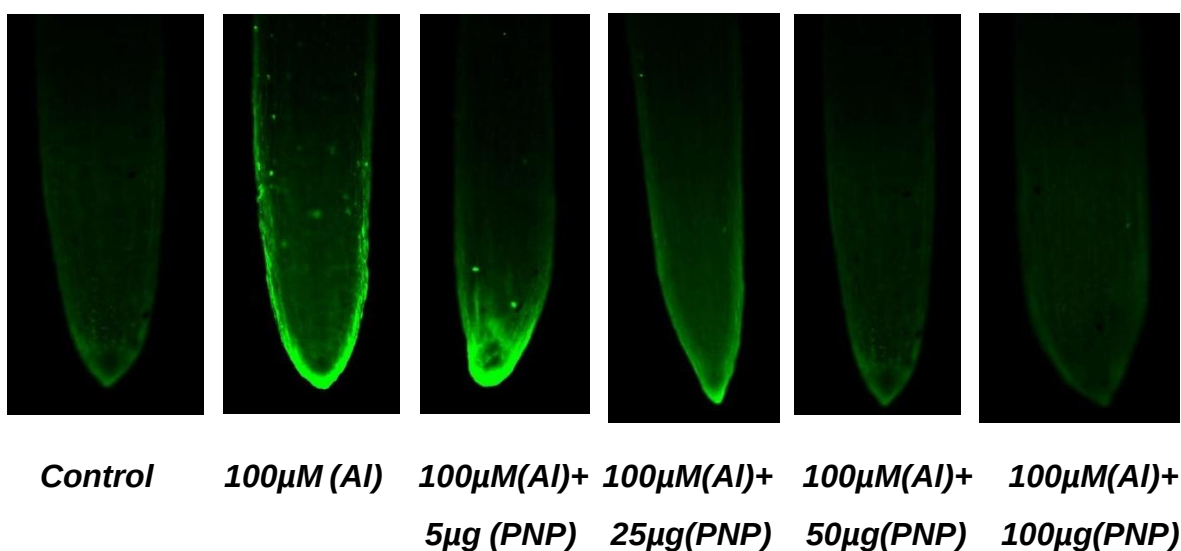


**Fig 5.8 Germination rate of Blackgram treated with PNP under Aluminium stress**

## 9.1 RESULTS FOR ROS ACCUMULATION

### 5.9.1 ROS accumulation on root tips of the seedlings

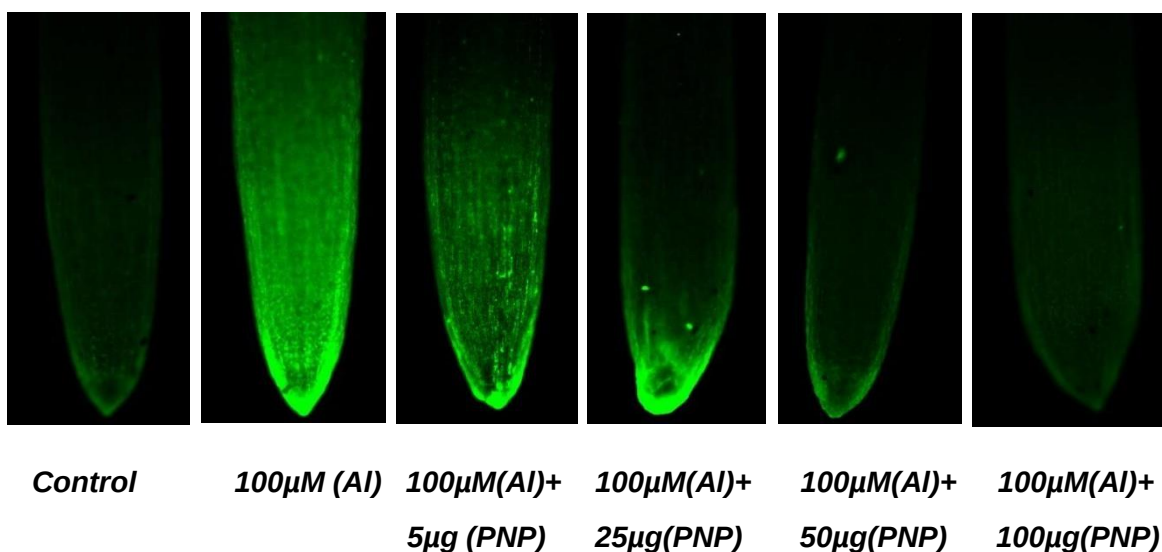
Root tips are stained with Dichlorodihydrofluorescein diacetate (DCFDA) to measure the ROS accumulation on the root tips in response to stress condition. DCFH-DA dye enables us to visualize the ROS accumulation in the root tips seedling. The bright fluorescence green in the root tips indicates the level of ROS accumulation in the tips. Unpaired seedlings grown under stress control show high level of ROS accumulation due to the induced stress damages in the roots of those seedlings. The root tips of seedlings primed with 100 $\mu$ g concentration have no ROS accumulation. The other concentrations (5 $\mu$ g, 25 $\mu$ g, 50 $\mu$ g) have a low level of ROS accumulation in the edges oh the root tips.



***Fig 5.9 ROS accumulation in root tips of Blackgram seedlings  
Treated with PNP***

### 5.9.2 Accumulation of aluminium on root tips of the seedlings

Morin dye enables us to visualize the accumulation of aluminium in the root tips of the black gram seedlings. The seedlings that are primed with paramylon show a dark green colour in the Confocal Microscopy which indicates the low-level accumulation of aluminium, Fluorescence green is observed in the root tips which indicates the high accumulation of aluminium. Thus, from the images we can infer the seed priming with paramylon controls and the seeds.



**Fig 5.10 Aluminium accumulation in root tips of Blackgram seedlings  
Treated with PNP**

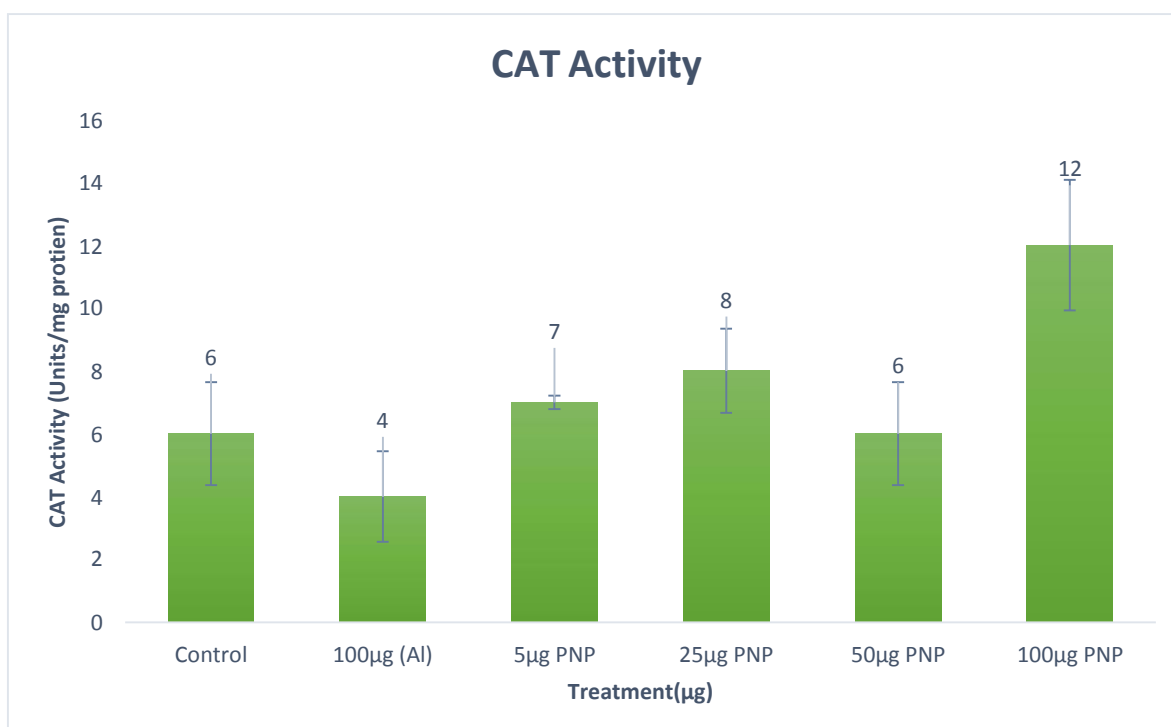
## 5.10. ANTIOXIDANT ENZYME ACTIVITY

### 5.10.1 Catalase Activity

CAT activity would indicate the ability of blackgram seedlings to scavenge hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a reactive oxygen species (ROS) produced under stress condition. Treatment with 100µM Al stress brought about a significant decrease in the activity of CAT when compared to control conditions. It was found that a significant increase in its activity under Paramylon nanoparticle treatment.

| BLACKGRAM | CAT ACTIVITY(Units/mg) |
|-----------|------------------------|
| CONTROL   | 6 ± 1.64               |
| Al STRESS | 4 ± 1.4                |
| 5µg PNP   | 7 ± 0.21               |
| 25µg PNP  | 8 ± 1.34               |
| 50µg PNP  | 6 ± 1.64               |
| 100µg PNP | 12 ± 2.081             |

**Table 5.7 CAT Activity of Blackgram seedling**



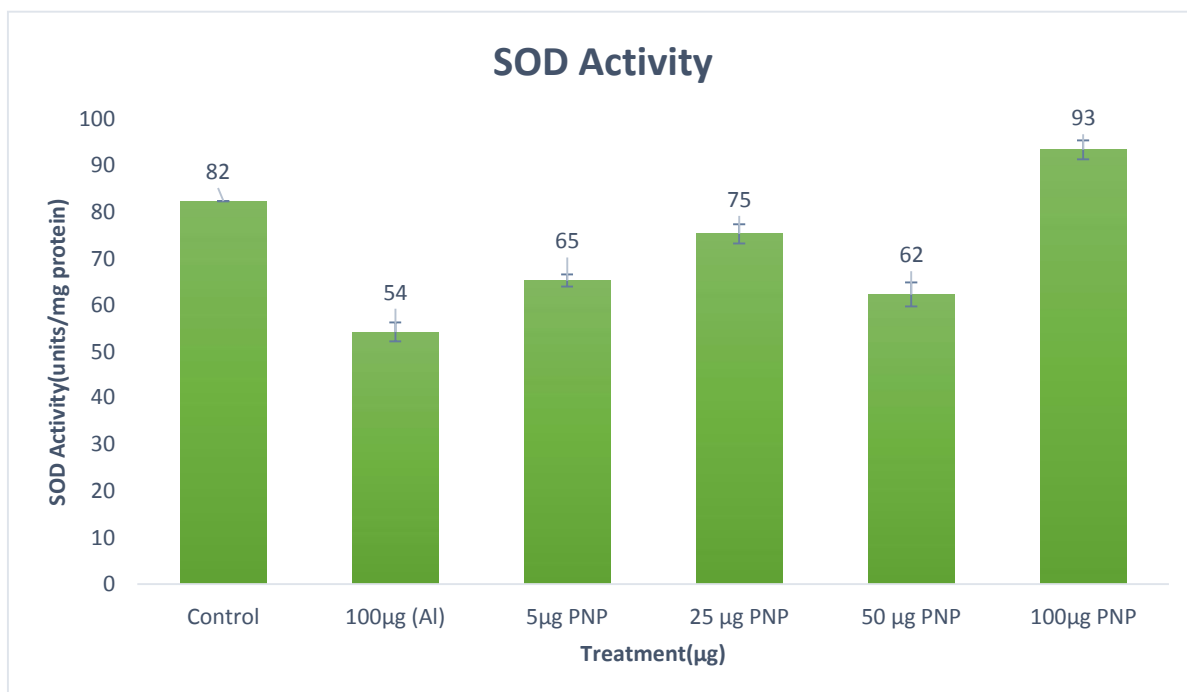
**Fig 5.11 Effect of PNP priming on CAT Activity of Blackgram under Aluminium stress**

### 5.10.2 Superoxide Dismutase(SOD) Activity

SOD activity would reflect the capacity of alfalfa to dismutase Superoxide radicals into oxygen(O<sub>2</sub>) and H<sub>2</sub>O<sub>2</sub>. Increased SOD activity would indicate enhanced ROS scavenging capacity and decreased damage to lipids of the plasma membrane under stress. Treatment with 100μM Al stress brought about a significant decrease in the activity of SOD when compared to control condition. It was found that a significant increase in SOD activity in control and slight increase in its activity under paramylon nanoparticle treatment.

| BLACKGRAM | SOD ACTIVITY(Units/mg) |
|-----------|------------------------|
| CONTROL   | 82 ± 2.052             |
| Al STRESS | 54 ± 1.29              |
| 5μg PNP   | 65 ± 2.081             |
| 25μg PNP  | 75 ± 2.581             |
| 50μg PNP  | 62 ± 2.051             |
| 100μg PNP | 93 ± 1.632             |

**Table 5.8 Effect of PNP priming on SOD Activity of Blackgram under Aluminium stress**



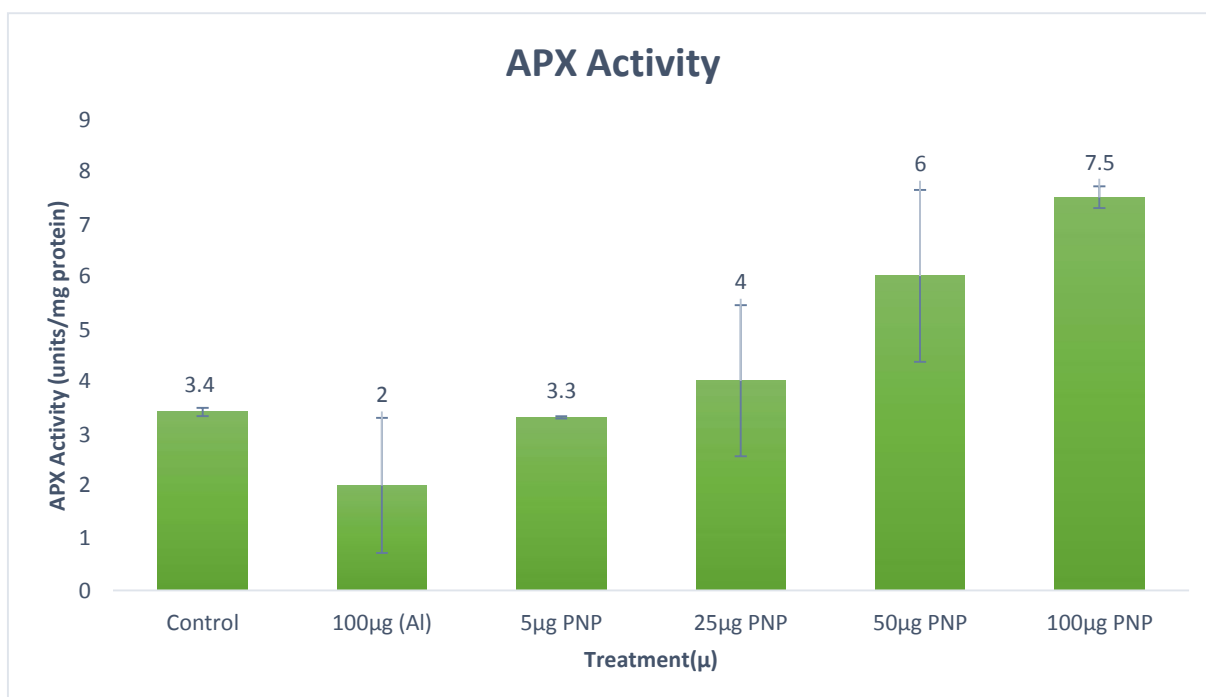
**Fig 5.12 Effect of PNP priming on SOD Activity of Blackgram under Aluminium stress**

### 5.10.3 Ascorbate Peroxidase(APX) Activity

APX activity would show the ability of blackgram to detoxify  $H_2O_2$  by utilizing ascorbate as a specific electron donor, thereby preventing oxidative stress. Elevated APX activity would imply improves ROS scavenging and tolerance to aluminium stress. Treatment with  $100\mu M$  Al stress brought about a significant decrease in the activity of APX when compared to control conditions. It was found that a significant increase in APX activity in control and slight increase in its activity under Paramylon nanoparticle treatment.

| BLACKGRAM      | APX ACTIVITY(Units/mg) |
|----------------|------------------------|
| CONTROL        | $3.4 \pm 0.081$        |
| Al STRESS      | $2 \pm 1.29$           |
| $5\mu g$ PNP   | $3.3 \pm 0.024$        |
| $25\mu g$ PNP  | $4 \pm 1.44$           |
| $50\mu g$ PNP  | $6 \pm 1.64$           |
| $100\mu g$ PNP | $7.5 \pm 0.208$        |

**Table 5.9 Effect of PNP priming on APX Activity of Blackgram under Aluminium stress**



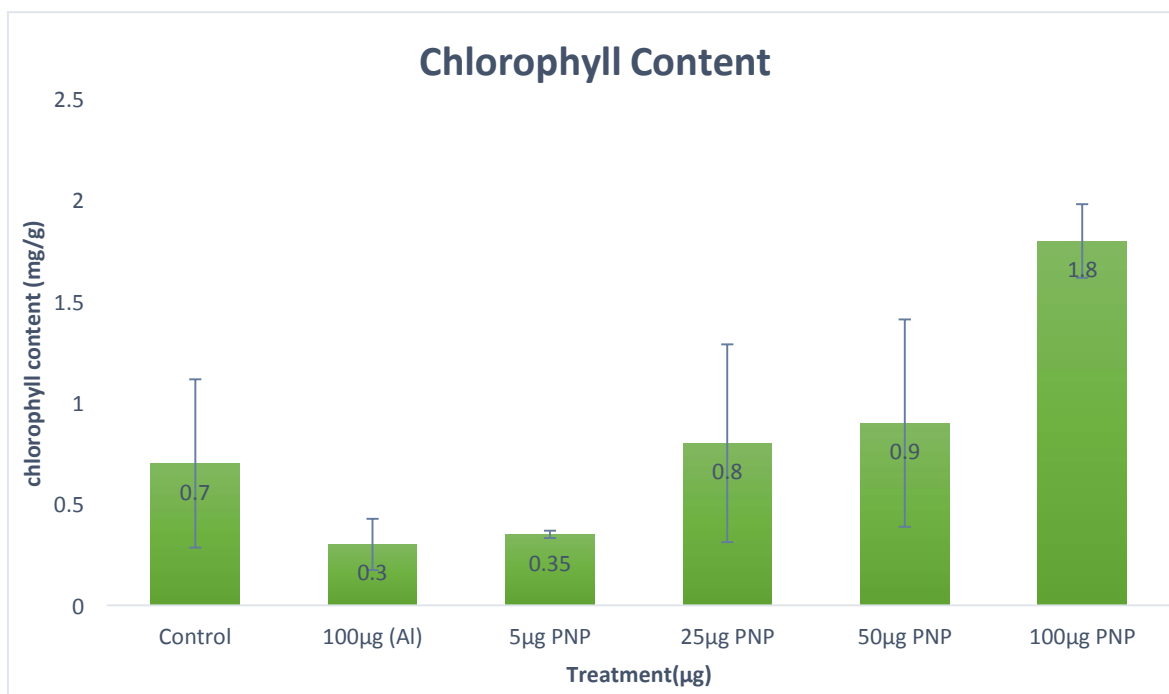
**Fig 5.13 Effect of PNP priming on APX Activity of Blackgram under Aluminium stress**

## 5.11 CHLOROPHYLL CONTENT

Chlorophyll, a green pigment responsible for photosynthesis, plant tissue. In this experiment, measuring chlorophyll content can help us to assess how the presence of Paramylon nanoparticle affects the ability of blackgram plants to photosynthesize and grow under aluminium stress. The highest chlorophyll content was found in the 100 $\mu$ M (Al) stress + 100 $\mu$ g (NPN) concentrations and the lowest chlorophyll content was observed under stress condition.

| BLACKGRAM       | CHLOROPHYLL CONTENT(mg/g) |
|-----------------|---------------------------|
| CONTROL         | 0.7 $\pm$ 0.416           |
| Al STRESS       | 0.3 $\pm$ 0.126           |
| 5 $\mu$ g PNP   | 0.35 $\pm$ 0.018          |
| 25 $\mu$ g PNP  | 0.8 $\pm$ 0.489           |
| 50 $\mu$ g PNP  | 0.9 $\pm$ 0.513           |
| 100 $\mu$ g PNP | 1.8 $\pm$ 0.182           |

**Table 5.10 Effect of PNPpriming on chlorophyll content of Blackgram under Aluminium stress**



**Fig 5.14 Effect of PNPpriming on chlorophyll content of Blackgram under Aluminium stress**

## CHAPTER-6

### SUMMARY AND CONCLUSION

This study demonstrates the potential of *Paramylon* nanoparticles extracted from *Euglena gracilis* in mitigating the adverse effect of aluminium stress on the germination *Vingo mungo* seeds. Paramylon nanoparticle was subjected to FTIR and SEM analysis. So the primed seeds contain high chlorophyll content at 100µg nanoparticle concentration. Through this experimentation, we observed a significant improvement in germination rates and seedling growth when primed with *Paramylon* nanoparticles compared to unprimed seeds under aluminium stress condition. Also seed priming as an effective result in overcoming abiotic stress conditions. The ROS result demonstrates that lowest ROS accumulation was found in primed seeds under 5µg, 25µg, 50µg concentration of Paramylon nanoparticle. DCFH-DA dye used to analyze ROS accumulation and Morin dye is used to analyze Aluminium accumulation. Also the primed seeds contain high chlorophyll content at 100µg nanoparticle concentration. The higher level of Anti-oxidant enzymes such as CAT, SOD and APX are higher in the seeds that are primed with PNP and compared to control and decreased activity under 100µg PNP concentration. These findings suggest that *Paramylon* nanoparticles could serve as a promising tool for enhancing crop resilience to environmental stressors, such as aluminium stress. Therefore, from overall study, we can understand that the seed priming of paramylon nanoparticle in blackgram seedling can effectively overcome the applied Aluminium stress condition and positively result in the overall growth characteristics of the blackgram (*vigna mungo*) seedlings.

## REFERENCES

- Achary, V. M. M., Jena, S., Panda, K. K., & Panda, B. B. (2018). Aluminium induced oxidative stress and DNA damage in root cells of *Allium cepa* L. *Ecotoxicology and environmental safety*, 70(2), 300-310.
- Aftab, T., Khan, M. M. A., Idrees, M., & Naeem, M. (2016). Effects of aluminium exposures on growth, photosynthetic efficiency, lipid peroxidation, antioxidant enzymes and artemisinin content of *Artemisia annua* L. *Journal of Phytology*, 2(8).
- Alvarez, I., Sam, O., Reynaldo, I., Testillano, P. S., Risueño, M. C., & Aria, M. (2015). Morphological and cellular changes in rice roots (*Oryza sativa* L.) caused by Al stress.
- Alvim, M. N., Ramos, F. T., Oliveira, D. C., Isaias, R. M. S., & Franca, M. G. C. (2019). Aluminium localization and toxicity symptoms related to root growth inhibition in rice (*Oryza sativa* L.) seedlings. *Journal of biosciences*, 37(Suppl 1), 1079-1088.
- Al-waibi, M., & Mohammad, F. (2015). Role of nanoparticles in plants. *Nanotechnol Plant Sci*, 1, 19-35.
- Banerjee, A., & Roychoudhury, A. (2017). Abiotic stress, generation of reactive oxygen species, and their consequences: an overview. *Reactive Oxygen Species in Plants: Boon or Bane-Revisiting the Role of ROS*, 23-50.
- Bhuja, P., McLachlan, K., Stephens, J., & Taylor, G. (2015). Accumulation of 1, 3- $\beta$ -D-glucans, in response to aluminum and cytosolic calcium in *Triticum aestivum*. *Plant and cell physiology*, 45(5), 543-549.
- Boscolo, P. R., Menossi, M., & Jorge, R. A. (2016). Aluminum-induced oxidative stress in maize. *Phytochemistry*, 62(2), 181-189.
- Cançado, G. M. A., Loguercio, L. L., Martins, P. R., Parentoni, S. N., Paiva, E., Borém, A., & Lopes, M. A. (2015). Hematoxylin staining as a phenotypic index for aluminum tolerance selection in tropical maize (*Zea mays* L.). *Theoretical and Applied Genetics*, 99, 747-754.

- Chandra, J., Parkhey, S., Varghese, D., Sershen, Varghese, B., & Keshavkant, S. (2020). Aluminium rhizotoxicity in *Cicer arietinum*. *Russian Journal of Plant Physiology*, 67, 945-952.
- Chowra, U., Yanase, E., Koyama, H., & Panda, S. K. (2017). Aluminium-induced excessive ROS causes cellular damage and metabolic shifts in black gram *Vigna mungo* (L.) Hepper. *Protoplasma*, 254, 293-302.
- Čiamporová, M. (2000). Diverse responses of root cell structure to aluminium stress. *Plant and Soil*, 226(1), 113-116.
- Dey, S., Chowardhara, B., Regon, P., Kar, S., Saha, B., & Panda, S. K. (2022). Iron deficiency in blackgram (*Vigna mungo* L.): redox status and antioxidant activity. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 156(2), 411-426.
- Dhoke, S. K., Mahajan, P., Kamble, R., & Khanna, A. (2013). Effect of nanoparticles suspension on the growth of mung (*Vigna radiata*) seedlings by foliar spray method. *Nanotechnology development*, 3(1), e1-e1.
- Dikilitas, M., Simsek, E., & Roychoudhury, A. (2020). Role of proline and glycine betaine in overcoming abiotic stresses. *Protective chemical agents in the amelioration of plant abiotic stress: biochemical and molecular perspectives*, 1-23.
- Farooqui, A. R. E. E. B. A., Tabassum, H. E. E. N. A., Ahmad, A. S. A. D., Mabood, A. B. D. U. L., Ahmad, A. D. N. A. N., & Ahmad, I. Z. (2016). Role of nanoparticles in growth and development of plants: A review. *Int J Pharm Bio Sci*, 7(4), 22-37.
- Feizi, H., Rezvani Moghaddam, P., Shahtahmassebi, N., & Fotovat, A. (2012). Impact of bulk and nanosized titanium dioxide (TiO<sub>2</sub>) on wheat seed germination and seedling growth. *Biological trace element research*, 146, 101-106.
- Foy, C. D. (1988). Plant adaptation to acid, aluminum-toxic soils. *Communications in Soil Science and Plant Analysis*, 19(7-12), 959-987.
- Guo, D., Xie, G., & Luo, J. (2013). Mechanical properties of nanoparticles: basics and applications. *Journal of physics D: applied physics*, 47(1), 013001.

Guo, P., Qi, Y. P., Cai, Y. T., Yang, T. Y., Yang, L. T., Huang, Z. R., & Chen, L. S. (2018). Aluminum effects on photosynthesis, reactive oxygen species and methylglyoxal detoxification in two Citrus species differing in aluminum tolerance. *Tree physiology*, 38(10), 1548-1565.

Hafeez, A., Razzaq, A., Mahmood, T., & Jhanzab, H. M. (2015). Potential of copper nanoparticles to increase growth and yield of wheat. *J Nanosci Adv Technol*, 1(1), 6-11.

Hossain, Z., Yasmeen, F., & Komatsu, S. (2020). Nanoparticles: synthesis, morphophysiological effects, and proteomic responses of crop plants. *International Journal of Molecular Sciences*, 21(9), 3056.

Hung, W.-C., Huang, D.-D., Chein, P.-S., Yeh, C.-M., Chen, P.-Y., Chi, W.-C., Huang, H.-J., 2007. Protein tyrosine dephosphorylation during copper-induced cell death in rice roots. *Chemosphere* 69, 55-62. Ishida, A., Ookubo, K., Ono, K., 2016.

Imtiaz, H., Shiraz, M., Mir, A. R., & Hayat, S. (2022). Green synthesis of nanoparticles and their effect on plant growth and development: a review. *Plant Archives (09725210)*, 22(1).

Jha, Z., Behar, N., Sharma, S. N., Chandel, G., Sharma, D. K., & Pandey, M. P. (2011). Nanotechnology: prospects of agricultural advancement. *Nano Vision*, 1(2), 88-100.

Jones, D.L., Blancaflor, E.B., Kochian, L.V., Gilroy, S., 2016. Spatial coordination of aluminium uptake, production of reactive oxygen species, callose production and wall rigidification in maize roots. *Plant Cell Environ.* 29, 1309-1318.

Kannan, P., Arunachlam, P., Prabukumar, G., & Prabhakaran, J. (2014). Response of blackgram (*Vigna mungo* L.) to multi-micronutrient mixtures under rainfed Alfisol. *Journal of the Indian Society of Soil Science*, 62(2), 154-160.

Kaphle, A., Navya, P. N., Umapathi, A., & Daima, H. K. (2018). Nanomaterials for agriculture, food and environment: applications, toxicity and regulation. *Environmental chemistry letters*, 16, 43-58.

Kaur, R., Sharma, M., Ji, D., Xu, M., & Agyei, D. (2019). Structural features, modification, and functionalities of beta-glucan. *Fibers*, 8(1), 1.

- Khan, Z., & Upadhyaya, H. (2019). Impact of nanoparticles on abiotic stress responses in plants: an overview. *Nanomaterials in plants, algae and microorganisms*, 305-322.
- Kochian, L.V., 2015. Cellular mechanisms of aluminium toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46, 237-260.
- Ma, J. F. (2017). Syndrome of aluminum toxicity and diversity of aluminum resistance in higher plants. *International review of cytology*, 264, 225-252.
- Martínez-Ballesta, M., Gil-Izquierdo, Á., García-Viguera, C., & Domínguez-Perles, R. (2018). Nanoparticles and controlled delivery for bioactive compounds: Outlining challenges for new “smart-foods” for health. *Foods*, 7(5), 72.
- Mobin, M. (2013). Effects of cadmium-induced oxidative stress on growth and nitrogen assimilation in blackgram [*Vigna mungo* (L.) Hepper]. *Journal of Agricultural Sciences, Belgrade*, 58(1), 31-39.
- Murali Achary, V. M., & Panda, B. B. (2010). Aluminium-induced DNA damage and adaptive response to genotoxic stress in plant cells are mediated through reactive oxygen intermediates. *Mutagenesis*, 25(2), 201-209.
- Ohki, K. (2015). Aluminum stress on sorghum growth and nutrient relationships. *Plant and Soil*, 98, 195-202.
- Panda, S. K., & Matsumoto, H. (2010). Changes in antioxidant gene expression and induction of oxidative stress in pea (*Pisum sativum* L.) under Al stress. *Biometals*, 23, 753-762.
- Pandey, P., Srivastava, R. K., Rajpoot, R., Rani, A., Pandey, A. K., & Dubey, R. S. (2016). Water deficit and aluminum interactive effects on generation of reactive oxygen species and responses of antioxidative enzymes in the seedlings of two rice cultivars differing in stress tolerance. *Environmental Science and Pollution Research*, 23, 1516-1528.
- Ra, U. S., & Kb, I. L. A. M. U. R. U. G. U. (2017). EFFECT OF ALUMINIUM ON THE SURVIVAL OF Rhizobium ISOLATES UNDER STRESS CONDITION. *Indian J. Sci. Res*, 16(2), 19-26.

Ra, U. S., & Kb, I. L. A. M. U. R. U. G. U. (2017). EFFECT OF ALUMINIUM ON THE SURVIVAL OF Rhizobium ISOLATES UNDER STRESS CONDITION. *Indian J. Sci. Res*, 16(2), 19-26.

Roy, A.K., Sharma, A., Talukder, G., 2017. A time course study on the effect of aluminium on mitotic cell division in *Allium sativum*. *Mutat. Res.* 227, 221-226

Saha, B., Swain, D., Borgohain, P., Rout, G. R., Koyama, H., & Panda, S. K. (2020). Enhanced exudation of malate in the rhizosphere due to AtALMT1 overexpression in blackgram (*Vigna mungo* L.) confers increased aluminium tolerance. *Plant Biology*, 22(4), 701-708.

Silva, S., Pinto-Carnide, O., Martins-Lopes, P., Matos, M., Guedes-Pinto, H., & Santos, C. (2010). Differential aluminium changes on nutrient accumulation and root differentiation in an Al sensitive vs. tolerant wheat. *Environmental and Experimental Botany*, 68(1), 91-98.

Singh, S., Verma, A., & Dubey, V. K. (2012). Effectivity of anti-oxidative enzymatic system on diminishing the oxidative stress induced by aluminium in chickpea (*Cicer arietinum* L.) seedlings. *Brazilian Journal of Plant Physiology*, 24, 47-54.

Sivaguru, M., & Horst, W. J. (2015). The distal part of the transition zone is the most aluminum-sensitive apical root zone of maize. *Plant Physiology*, 116(1), 155-163.

Sultana, R., Choudhary, A. K., Pal, A. K., Saxena, K. B., Prasad, B. D., & Singh, R. (2014). Abiotic stresses in major pulses: current status and strategies. *Approaches to plant stress and their management*, 173-190.

Yamamoto, Y., Kobayashi, Y., & Matsumoto, H. (2018). Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. *Plant physiology*, 125(1), 199-208.

Yang, Z. B., Rao, I. M., & Horst, W. J. (2013). Interaction of aluminium and drought stress on root growth and crop yield on acid soils. *Plant and Soil*, 372, 3-25.