

SEED PRIMING EFFECT OF BETA-1,3-GLUCAN IN VIGNA
RADIATA (MUNG BEAN) GERMINATION AND SEEDLING IN
RESPONSE TO CADMIUM STRESS

Submitted in Partial Fulfillment of the requirement for the award of a Bachelor of
Science degree in Biotechnology

By

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

CATEGORY-1 UNIVERSITY BY UGC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **NANDHINI S (41770016)** who carried out the project entitled "**SEED PRIMING EFFECT OF BETA-1,3-GLUCAN IN VIGNA RADIATA (MUNG BEAN) GERMINATION AND SEEDLING IN RESPONSE TO CADMIUM STRESS**" under my supervision from January 2024 to April 2024.

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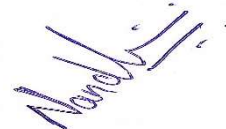
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DECLARATION

I, Nandhini. S (Reg. No. 41770016) hereby declare that the Project Report entitled „ SEED PRIMING EFFECT OF BETA-1,3-GLUCAN IN VIGNA RADIATA (MUNG BEAN) GERMINATION AND SEEDLING IN RESPONSE TO CADMIUM STRESS done by me under the guidance of Dr. S. M. INDUMATHI (Internal) and Dr. R. Srinivasan (External) at CENTRE FOR OCEAN RESEARCH, COL. Dr. JEPPIAAR RESEARCH PARK, SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY is submitted in partial fulfillment of the requirements for the award of a Bachelor of Science and Technology degree in Biotechnology

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ABSTRACT

Seed priming is a technique in which organic or inorganic substances are coated on the seeds to enhance their growth. Several seed priming techniques are used such as osmopriming, hydro-priming, chemical priming, solid-matrix priming, and hormonal priming. Seed priming majorly contributes to the overcoming of stress conditions by the seeds. Due to increasing soil pollution, heavy metals such as cadmium are found abundantly in the soil. Cadmium poses a serious threat to agriculture. Cadmium inhibits the growth of the seeds by inhibiting their entire biochemical processes. In this study, Paramylon, a linear unbranched beta-1,3 glucan extracted from the algae *Euglena gracilis* cell wall, is primed to the Mung bean (*Vigna radiata*) seeds to overcome the Cadmium stress condition. The morphological traits such as root and shoot length, fresh and dry weight, germination rate and seedling vigour index are observed for the Mung bean seedlings that are primed and grown under different concentrations of cadmium stress (0.5mM and 1mM). Also, the accumulation and ROS and cadmium in the root tips of the seedlings are visualized by staining the tips with Dichlorodihydrofluorecein diacetate dye and leadmium green dye respectively. The Total chlorophyll content from the extract of the seeds is also evaluated to determine the impact of seed priming. The higher level of Anti-oxidant enzymes such as CAT, APX and SOD are higher in the seeds that are primed with Paramylon concentration of 1mg/ml. Therefore, from the overall study, we can understand that the seed-priming of Paramylon on Mung bean seeds can effectively overcome the applied Cadmium stress condition and positively result in the overall growth characteristics of the Mung bean (*Vigna radiata*) seedlings.

Keywords: Seed priming, Paramylon, Mung bean, Cadmium stress, Dichlorodihydrofluorecein diacetate dye, Leadmium green dye.

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LIST OF ABBREVIATIONS

%	Percentage	Millilitre
µl	Microlitre	Na ₂ EDTA nm
APX	Ascorbate Peroxidase	
CAT	Catalase	
Cd cm	Cadmium	
CNP	Centi meter	
DCFH-DA	Chitosan Nanoparticle Particle	
EGP	2',7' – Dichlorodihydrofluorecein diacetate dye	
FTIR M	Euglena gracilis Paramylon	
mg ml	Fourier Transfer Infrared	
mM	Molar	
	Milligrams	
P	Ethylenediaminetetraacetic acid disodium salt dihydrate	
PEG	Nanometer	
ROS rpm	Paramylon	
SDS	Poly Ethylene Glycol	
SEM	Reactive Oxygen Species	
SOD	Rotations per minute	
	Sodium Dodecyl Sulphate	
	Scanning Electron Microscopy	
	Superoxide Dismutase	

Milli molar

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CHAPTER – 1

INTRODUCTION

Plants that are part of the Fabaceae family are called Legumes. One of the most commonly used and well-known legumes is Mung bean (*Vigna radiata*). Mung Bean is an annual legume grown mostly in semi-arid regions during summer seasons. It has been cultivated in India since pre-historic times and also in Southern and Eastern Asia, Central Africa, parts of China, South and North America and Australia for its protein-rich grains (Dahiya et al., 2015). It helps improve soil fertility by fixing nitrogen, leading to sustainable agriculture. Mung bean is a suitable plant for toxicity tests because of its sensitivity to metal contamination, easy-to-grow character and cultivatable in a short span of time.



Fig: 1.1 Mung bean (*Vigna radiata*) seeds

In this Modern World, Heavy metals threaten human health and the environment. They are accumulated in the soil by discharges from urban and industrial wastes as well as poor agricultural practices (Elleuch et al., 2013). Cadmium is one of those heavy metals which is more persistent and toxic even at low concentrations. Cd has a long biological half-life of 17 to 30 years (Zayneb et al., 2015). The minute amount of this toxic element is capable enough to damage the vital functions of plants such as germination, seedling growth and photosynthesis (Abhilasha Shourie, 2022).

Cadmium stress in *Sorghum bicolour* seeds reduced the storage mobilisation process (Kalai et al., 2015). Wang et al., 2012 investigated that root growth was decreased with an increase in the concentration of cadmium in *Lactuca sativa*. Membrane damage was reported in *Oryza sativa* and *Triticum aestivum* under cadmium stress (Qin et al, 2020).

Severe Retardation in germination has been reported in several plant families such as Fabaceae: pea (*Pisum sativum*) (Majeed et al, 2019), Asteraceae: sunflower (*Helianthus annuus*) (Jadia & Fulekar, 2009), Brassicaceae: canola (*Brassica napus*) (Moosavi et al, 2012). Anwar et al. observed that the mobilisation and translocation of hydrolysed enzymes and sugars are also inhibited by cadmium. Membrane damage was reported in *Oryza sativa* and *Triticum aestivum* under cadmium stress. Cadmium uptake is also likely to affect Fe (Iron) accumulation that results in Leaf Chlorosis – “heavy metal-induced Fe-deficiency chlorosis” (Qin et al., 2020).

Seed priming is a successful method in which seeds are hydrated (controlled hydration or uncontrolled hydration) and dried to the original moisture content. Priming is a lowcost and eco-friendly technique to enhance seed germination. It allows pre-germinative metabolism to proceed but prevents radicle emergence (Soughir et al., 2013). Theophrastus observed that cucumber seeds, when soaked in water (Hydropriming) resulted in uniform and faster germination as compared to unprimed seeds (Theophrastus, Enquiry into plants book VII, I.6). The exogenous application of ascorbic acid and salicylic acid enhanced the wheat seeds to grow under salinity stress (Bahrani A & Pourezza J, 2012, Afzal. I et.al 2006). Fenugreek seeds primed with Ginger extracts resulted in the highest germination percentage, seedling length and speed of germination (Mounika et al., 2021).

Chemical and Physical factors such as osmotic and water potential, Priming agent, duration, temperature, presence or absence of light, aeration and seed condition play an important role in determining the priming success, germination rate, seedling vigour and further plant development. Hydropriming is the technique in which the seeds are soaked in pure water and are redried to their original moisture content prior to sowing. There is no use of chemical substances in this method, hence it is eco-friendly and of low cost. However, the uncontrolled water uptake by seeds is the major disadvantage of Hydropriming. The desired level of seed hydration can be ensured by accurate treatment duration, temperature and water volume used in hydropriming and to prevent radicle protrusion.

In Osmo-priming, the seeds are soaked in an osmotic solution that has low water potential instead of pure water. The water enters the seeds slowly with gradual seed imbibition and activation of early phases of germination, preventing radicle protrusion. The compounds used for osmo-priming are Polyethylene Glycol (PEG), mannitol, sorbitol, Glycerol, and inorganic salts such as NaCl, KCl, KNO₃, K₃PO₄, KH₂PO₄, MgSO₄ and CaCl₂. Priming with salt solution is known as Halopriming.

In the Solid-matrix priming method, the seeds are mixed and incubated with a wet solid water carrier for a certain period. The use of a solid medium allows seeds to hydrate slowly and stimulates the natural imbibition process occurring in the soil. Vermiculite, peat moss, charcoal, sand, and clay are some of the substrates used for seed germination.

The seeds are primed with plant growth regulators such as Absciscic acid, Auxin, Gibberellins, Kinetin, Ethylene, Salicylic acid, and Polyamines. Hormonal priming directly impacts seed metabolism and germination. The application of Absciscic acid on rice seeds has enhanced the growth rate, biomass accumulation, and root formation of rice under alkaline stress. (Wei et al., 2015). The scientists observed that wheat seeds primed with Cytokinin showed enhanced chlorophyll formation thereby improving photosynthetic rate, membrane stability, and regulating ionic levels under severe drought stress (Iqbal et al., 2006).

In Biopriming, the seeds are pre-soaked with beneficial microorganisms. This method combines both biological agent(microbes) and physiological soaking (seed hydration) phase (Waqas et al., 2019). Seeds are usually coated with rhizome microorganisms, an environmentally beneficial ecological approach that enhances plant growth by promoting nutrients or by protecting seedlings against the soil or seed-borne plant pathogenic organisms (S.R. Prasad et al., 2016).

Chemical seed priming is a physiological technology that involves exposing seeds to a stimulating factor. Some chemicals used for seed priming include Natural substances – organic acids, plant extracts, chitosan, and polyamines. Synthetic compounds – Sodium nitroprusside, Sodium hypochlorite. Other chemicals – Selenium, Ethanol, CuSO₄, ZnSO₄, KNO₃. Chemical priming can make plants more tolerant to stress exposure. For example, rice seedlings grown from calcium chloride (CaCl₂) pre-treated seeds displayed enhanced resistance to the rice blast fungus and the rice bacterial pathogen. Priming activates the germination process, which reduces the shelf life of primed seeds compared to unprimed seeds. Seed Priming with polysaccharides plays a significant role in protecting the reactions in plants against various stresses.

Glucans is the common name given to a group of polysaccharides that are classified based on the interchain linkages as either alpha or beta-linked. The common structure comprises a main chain of Beta-(1,3)-Glucopyranosyl units in nonrepeating but non-random order with side chains of varying length. The degree of branching and the length of the side chains can vary between different types of betaglucans. This variability affects their solubility, viscosity, and functional properties. Beta-glucans are found in mushrooms, yeasts, barley, oats, wheat and rye. Betaglucans derived from oats and barley typically have mixed linkages, including $\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$, whereas those from fungi and yeast often have a predominantly $\beta(1\rightarrow3)$ -linked backbone.

According to Inui et al., 1997., Beta-glucans are active elicitors of resistance responses in various plants and in addition to laminarhexose, stimulate protection of cell suspensions of rice. Hence, coating seeds with beta-glucans may enhance seed germination and early seedling development. This can lead to improved crop establishment and overall yield. Beta-glucans have been studied for their ability to induce systemic acquired resistance (SAR) in plants. Beta-glucans have been investigated for their potential to enhance stress tolerance in plants. They may help crops withstand environmental stressors such as drought, salinity, and extreme temperatures. This can be particularly beneficial in regions prone to challenging growing conditions.

SAR is a mechanism by which plants enhance their resistance to a broad spectrum of pathogens. Applying beta-glucans to crops may help activate the plant's defence mechanisms, making them more resistant to diseases caused by bacteria, fungi, and viruses. Beta-glucans can act as bio-stimulants, promoting plant growth and development. They may enhance the efficiency of nutrient utilization and improve overall crop productivity.

Paramylon (a Greek-derived term. Para – similar; Amylon - starch) is a linear, unbranched Beta-1,3-Glucan polysaccharide with high molecular weight. Gottlieb (1856) isolated the starch-like granules from *Euglena* species of algae. He showed that they were composed of carbohydrates which are isomeric with starch and not stained with iodine. Paramylon is a storage polysaccharide of the *Euglena* genus. This polysaccharide is present in the Chloroplasts of *Euglena gracilis* and when there is an unfavourable condition, this stored paramylon acts as an energy source. When *Euglena* is grown under heterotrophic conditions, it can accumulate large amounts of this reserve polysaccharide nearly up to 95% of its cell mass (Russo et al., 2016).

Paramylon has high crystallinity. Paramylon grains can be small and numerous showing great diversity in number and forms within Euglenoids. Paramylon can be easily isolated from the cells simply by disrupting the cell walls. Hence the isolation cost is low compared to other glucose polysaccharides. Due to its unbranched, linear structure, it can easily interact with PPRs on cell membranes of the plant which influences their growth pattern. Paramylon can fight against the plant's abiotic stress condition by enhancing the diffusion of air to the carboxylation sites (Andrea et al., 2017).

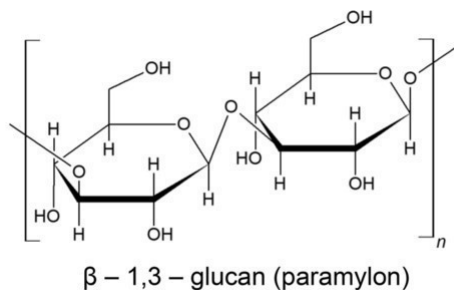


Fig: 1.2 Structure of Paramylon

CHAPTER 2

LITERATURE SURVEY

2.1 ANALYSIS OF PARAMYLON

Analysis of paramylon grains from different Euglenoid species in vivo and in vitro using scanning electron and light microscopy. A diversity of paramylon types is found within the genus *Euglena*. SEM images show that ellipses and links or short rods are present in nearly all species. For *Euglena gracilis*, in addition to ellipses and short rods, many *Euglena* species have chloroplasts with centrally located pyrenoids that are capped on both sides with shallow cup-shaped paramylon grains. Another work's target is to improve the functional characteristics of EGP by hydrogen peroxide (H_2O_2) degradation and carboxymethylated modification. The results showed that H_2O_2 and carboxymethylated modification increased the water solubility increased by 9% and 85% respectively. EGP's adsorption capacity for oil, dye and metal ions was increased about 2 times after carboxymethylated treatment. (Monfil et al., 2011, Liwei et al, 2022).

This study aimed to investigate the diversity of paramylon grains, a characteristic feature of euglenoid cells, across various species both in vivo and in vitro. Through the examination of over 1000 light microscopy images and scanning electron microscopy analysis of 11 representative species, including *Colacium vesiculosum*, *Cryptoglana skujae*, *Discoplastis spathirhyncha*, *Euglena gracilis*, *Lepocinclis acus*, *L. ovum*, *Monomorphina pyrum*, *Phacus orbicularis*, *P. pleuronectes*, *Strombomonas borysthensiensis*, and *Trachelomonas ellipsoidalis*, the study aimed to establish a consistent terminology for describing paramylon grain types and evaluate their potential phylogenetic utility. Distinct morphological categories for paramylon grains were identified, including disk, ellipse, pyrenoid cap, plate, bobbin or rod, with further subcategories delineated within each type. These categories were then applied to assess the diversity of paramylon grains within genera and major euglenoid lineages. The study revealed significant variation in paramylon grain morphology both within and among species, spanning

a range of sizes from large to small grains. The findings suggest that paramylon grain morphology can serve as a useful tool for supporting major clades and generic relationships within the euglenoid lineage. Furthermore, Paramylon characteristics may offer insights into the taxonomic placement of euglenoids that are currently inaccessible for sequencing analysis (Monfil et al., 2011).

The influence of growth conditions on paramylon, a β -1,3-glucan reserve carbohydrate, was investigated in both the photosynthetic wild-type and a nonphotosynthetic WZSL mutant of the unicellular flagellate *Euglena gracilis*. Paramylon holds promise for various applications in human and veterinary medicine as an immunostimulant and immunopotentiator. Results revealed that the synthesis of this reserve carbohydrate is predominantly influenced by growth conditions, specifically light exposure or darkness. Under dark conditions and with glucose serving as the carbon source, the WZSL mutant accumulated the highest amount of paramylon, accounting for 90% of its dry weight. These findings prompt discussion regarding the potential utilization of both strains as alternative sources of β -glucan and underscore the significance of the chloroplast compartment in paramylon synthesis (Barsanti et al., 2001).

2.2 EFFECT OF CADMIUM ON SEEDLINGS GROWTH

The effect of cadmium on mung bean seedlings was studied. The results showed that Cd affects mung bean root and shoot length. When applying EDTA as a chelating agent, it detoxified Cd while also promoting root and shoot growth in the mung bean seedlings. The parameters of mung bean under Cd stress, such as reduction in the growth of root and shoot, changes in the root tip brown colour and the quality of root cell damage can be used as a bio-indicator for detecting Cd and monitoring Cd levels in the environment. The barley seeds are pretreated with Salicylic acid and exposed to Cd stress. The germination percentage has seemed to be reduced in higher Cd concentrations. The pre-treatment of barley seeds with salicylic acid caused less toxicity on the mitotic index and greater amylase and phosphatase activity under Cd stress. (Chanya et al., 2022, Kalai et al., 2015).

Two rice varieties with high and low tolerance of cadmium stress were examined. An increase in the cadmium concentration comparatively reduced the germination index, vigour index, radicle length and amylase activities. Also, when there is a higher concentration of cadmium stress there is greater inhibition of cell division was observed. The effect of cadmium stress on mung bean seed germination, plant growth and activities of hydrolytic enzymes were studied. The level of total soluble sugars and protein levels decreased, while the free amino level increased under cadmium stress. The Cd stress also inhibits the mobilization and translocation of hydrolysed sugars to an embryonic axis which results in the inhibition of seedling growth. The highest Cd concentration (100mgL^{-1}) caused a decrease in the germination parameters such as germination percentage, shoot length, root length, and fresh and dry biomass. (Jun-yu et al., 2008, Anwar et al., 2021).

Cd tolerance in plants is closely linked to the accumulation of Cd within plant tissues. Typically, plants with high Cd tolerance employ strategies to prevent excessive metal accumulation, thus averting the onset of toxicity symptoms. These strategies involve mechanisms such as restricting Cd uptake, enhancing Cd efflux from the cytosol, and reducing Cd transport across cell membranes as well as from roots to shoots. Cd-tolerant plants, especially hyperaccumulators, further mitigate Cd toxicity by minimizing its interaction with cellular organelles and modulating physio-biochemical mechanisms. These mechanisms include sequestering Cd at inactive sites like foliar trichomes and the cell wall, compartmentalizing Cd within vacuoles via tonoplast-located transporters, and chelating Cd in the cytosol using peptides such as phytochelatins, glutathione, oxalic acid, citric acid salts, and phosphoric acid. The capacity for Cd accumulation and tolerance varies among plant species. Studies by Shao et al. and Liu have highlighted significant differences in seedling growth and antioxidant enzyme activities among various Cd-tolerant rice genotypes. Similarly, our previous research revealed marked disparities in Cd tolerance and accumulation among rice genotypes, notably exemplified by the contrast between the Cd-tolerant Xiushui 110 and the Cd-sensitive Xiushui 11. This study aimed to elucidate the effects of cadmium on seed germination, plumule and

radicle growth, and seed amylase activities in these two rice varieties, thereby shedding light on the mechanisms of Cd toxicity in rice plants. Such insights could contribute to enhancing plant growth security in contaminated soil environments.

Another study investigated the impact of cadmium (Cd) exposure on the growth of mung bean (*Vigna radiata* (L.) R. Wilczek). Mung beans were cultivated in a Hoagland solution with varying Cd concentrations (0, 0.1, 0.3, and 0.5 mg/L) for a duration of 5 days. Results demonstrated a significant reduction in both root and shoot lengths of mung bean plants exposed to Cd, with the severity of the effect correlating with Cd concentrations. To assess cell death in mung bean roots, Evan's blue staining was employed. Findings revealed a concentration-dependent increase in dead cells, particularly within the root cap zone, in Cd-exposed mung beans. The uptake of Evan's blue dye by Cd-exposed mung beans ranged from 1.5612 ± 0.5417 to 8.0850 ± 2.6336 mg/L across the different Cd concentrations. This suggests the potential utility of Evan's blue staining as a biomarker for indicating Cd contamination in water. Additionally, the study explored the effectiveness of chelating agents, particularly EDTA, in mitigating Cd contamination. Results indicated the potential of EDTA as a treatment agent for Cd removal, with observed positive effects on plant growth in areas contaminated with Cd. (Phromchaloem & Kumkaeo., 2022).

2.3 EFFECT OF SEED-PRIMING ON SEEDLING GROWTH

Seed priming is a preparatory technique applied before sowing, aimed at inducing a physiological state in seeds that enhances their germination efficiency. Typically, primed seeds undergo initial imbibition, initiating the first stage of germination without radical protrusion through the seed coat. These seeds retain their desiccation tolerance after treatment and can be stored until final sowing. Upon subsequent germination, primed seeds display accelerated and synchronized germination, yielding seedlings that are often more robust and resilient to environmental stresses compared to those from untreated seeds. Various methods

of priming exist, including soaking seeds in specified amounts of water or limiting imbibition time. Osmopriming utilizes osmotic agents such as polyethylene glycol (PEG) to control the imbibition rate, while halopriming involves the use of specific salts. Hormopriming, on the other hand, employs plant growth regulators to induce priming effects. Additionally, physical treatments such as UV exposure, and cold or heat shocks, also contribute to improved germination, suggesting that priming effects extend beyond mere imbibition. Enhanced comprehension of the metabolic processes occurring during priming treatments and subsequent germination stages can facilitate more effective utilization of this cost-effective technology (Stanley et al., 2016).

The mung bean seeds are primed with chitosan on different water potential levels induced by PEG. The germination and seedlings parameters are estimated. The level of water stress affects the morphological and physio-chemical characteristics of the seedling. PEG-induced water stress significantly reduced the Relative water content, vigour index and Chlorophyll content in the seedlings. Meanwhile, the chitosan-primed seedlings seem to show improvements in terms of morpho-physiological and biochemical attributes under PEG-induced drought stress conditions.

(Akash Hidangmayum & Padmanabh Dwivedi, 2022).

Abiotic stress affecting crop production in arid and semi-arid regions in the world is Salinity stress. Seed priming is a successful technique used to improve the emergence of seedlings. In this study, NaCl is primed on fenugreek seeds in tolerance to salinity stress. The NaCl-primed seeds were observed to have improved germination characteristics in comparison to unprimed seeds. Thus, NaCl seed priming proved to be a useful application for plants in overcoming the stress condition. However, further studies should be conducted to analyse the molecular changes in inducing the growth parameters in primed seeds (Soughir et al., 2013). Chitosan is a type of Beta-1,3-glucan. Priming with Chitosan nanoparticles on wheat seeds was studied. Chitosan NP has a promising effect on the seedling growth of wheat even at lower concentrations (10fold). Also, the

absorption of CSNP on the surface of the seed is higher, which is one of the reasons why CSNP can promote the growth of seedlings at lower concentrations (Li et al.,2018).

The effects of exogenously applied Beta-glucan on the growth of *Oryza sativa* seedlings grown in vitro in NaCl stress treatment were studied. Growth characteristics, including shoot and root height, and fresh and dry weight of plants treated with Beta-glucan were improved. Thus, the application of Betaglucan exogenously on *Oryza sativa* can ameliorate the adverse effects of salt stress (Arshad et al., 2016). Because of its prolific growth, oilseed rape (*Brassica napus* L.) can be grown advantageously for phytoremediation of lands contaminated by industrial wastes. Therefore, the toxic effect of cadmium on the germination of oilseed rape, the capability of plants for cadmium phytoextraction, and the effect of exogenous application of plant growth regulators to mitigate the phytotoxicity of cadmium were investigated.

For the lab study of seedlings at an early stage, seeds were grown on filter papers soaked in different solutions of Cd₂ (0, 10, 50, 100, 200 and 400 μ M). In a greenhouse study, seedlings were grown in soil for 8 weeks, transferred to hydroponic pots for another 6 weeks of growth, and then treated with plant growth regulators and cadmium. Four plant growth regulators viz. jasmonic acid (12.5 μ M), abscisic acid (10 μ M), gibberellin (50 μ M) and salicylic acid (50 μ M); and three levels of Cd₂(0, 50 and 100 μ M) were applied. Data indicated that lower concentrations of Cd₂(10 μ M) promoted root growth, whereas the severe stresses (200 or 400 μ M) hurt the establishment of germinating seedlings. Plants treated with any of the tested plant growth regulators alleviated cadmium toxicity symptoms, which were reflected by more fresh weight, less malondialdehyde concentration in leaves and lower antioxidant enzyme activities. The application of abscisic acid to the plants cultivated in the medium containing 100 μ M Cd₂ resulted in significantly lower plant internal cadmium accumulation (Huabing et al.,2008).

In agricultural landscapes often characterized by fragility or limited resources, crop growth and development face significant challenges from both biotic and abiotic stresses. To address these issues, current research efforts are increasingly focused on short-term strategies aimed at enhancing crop performance in variable environments. Seed treatment or priming technology has emerged as a promising approach, albeit still gaining traction among resource-constrained farmers. Effective policy interventions could facilitate the widespread adoption of these low-cost techniques in developing nations, thereby maximizing the benefits of sustainable food production systems. Primed seeds exhibit heightened vigor and germination rates, crucial for robust seedling growth and successful crop establishment under stress conditions. This review aims to evaluate various seed priming techniques in terms of resource efficiency, crop productivity, costeffectiveness, and environmental considerations. Additionally, it delves into the physiological and biochemical mechanisms underlying seed priming, providing insights into its applications across diverse agroecosystems. A thorough exploration of priming technology's utility can enhance our understanding of adaptive resource management practices in agriculture (Devika et al., 2021).

Nano-priming is an innovative seed-priming technology that helps to improve seed germination, seed growth, and yield by providing resistance to various stresses in plants. Nano-priming is a considerably more effective method compared to all other seed-priming methods. The salient features of nanoparticles (NPs) in seed priming are to develop electron exchange and enhanced surface reaction capabilities associated with various components of plant cells and tissues. Nano-priming induces the formation of nanopores in the shoot and helps in the uptake of water absorption, activates reactive oxygen species (ROS) antioxidant mechanisms in seeds, and forms hydroxyl radicals to loosen the walls of the cells and acts as an inducer for rapid hydrolysis of starch. It also induces the expression of aquaporin genes that are involved in the intake of water and also mediates H_2O_2 , or ROS, dispersed over biological membranes. Nano-priming induces starch degradation via the stimulation of amylase, which stimulates seed germination.

Nano-priming induces a mild ROS that acts as a primary signalling cue for various signalling cascade events that participate in secondary metabolite production and stress tolerance. This review provides details on the possible mechanisms by which nano-priming induces breaking seed dormancy, promotion of seed germination, and their impact on primary and secondary metabolite production. In addition, the use of nano-based fertilizer and pesticides as effective materials in nano-priming and plant growth development were also discussed, considering their recent status and future perspectives (Shivraj et al., 2022).

The study conducted by Khan et al., 2021 aimed to investigate the potential mitigating effect of salicylic acid (SA) on cadmium (Cd) toxicity in mung bean plants, considering Cd accumulation as a significant environmental concern with adverse impacts on plant growth and development. Mung bean plants were subjected to varying Cd concentrations (0, 5, 10, and 15 mg Cd kg⁻¹ of soil), with exogenous application of SA at concentrations of 0, 10⁻⁶, and 10⁻³ M before flowering. Results revealed that Cd stress markedly impaired various growth-related parameters, including shoot length, root length, biomass, total soluble protein, total amino acids, relative water content, and photosynthetic pigments. Concurrently, Cd stress induced a notable increase in antioxidant levels, such as peroxidase (POD), ascorbate peroxidase (APX), ascorbic acid (AsA), and catalase (CAT), while also promoting the accumulation of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) contents. However, SA application significantly mitigated Cd-induced growth inhibition and oxidative stress by enhancing plant biomass and photosynthetic pigments. Additionally, SA bolstered antioxidant activities, mitigated Cd uptake, reduced membrane damage, and curtailed H₂O₂ and MDA accumulation. Overall, the study's findings suggest that exogenous SA application enhances mung bean seedling growth, augments antioxidant defence mechanisms, and mitigates oxidative damage under Cd-induced stress conditions.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To examine the effect of Seed-priming of Beta -1,3-glucan (Paramylon) on the Mung bean seeds under cadmium stress.

3.2 OBJECTIVES

- To coat the seeds with Paramylon of different concentrations (Seed priming).
- To incubate the seeds for germination under varying Cadmium stress conditions.
- Analysing the Germination rate, seedling growth and other characteristics of the Primed seeds in comparison with Unprimed seeds (control).
- To determine the enzyme activity in the treated Mung bean seedlings.

CHAPTER 4

MATERIALS AND METHODS

4.1 METHODOLOGY

4.1.1 Isolation of Paramylon

The materials required for isolation of Paramylon are SDS, Na₂EDTA and distilled water. Paramylon was extracted and purified from a 2-day-old *E. gracilis* WZSL mutant by suspending a pellet of cells in a solution containing 1% (w/v) SDS and 5% (w/v) Na₂EDTA. The suspension was incubated for 30 min at 37°C and the paramylon granules were recovered by centrifugation at 1000g for 10 min. The SDSNa₂EDTA treatment was repeated and the paramylon was washed twice with hot-glass distilled water (70°C). After the second wash, the granules were deposited on glass fibre filters, dried overnight at 90°C, and stored at room temperature. This was hydrated in deionized water (1.5% w/v) overnight at 4°C, then collected centrifugation at 1000g for 10 min, and dried overnight under vacuum at room temperature (Barsanti et al., 2019).



Fig 4.1 Paramylon isolated from *Euglena gracilis*

4.1.2 Characterization of Paramylon

4.1.2.1 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is a powerful imaging technique used to observe the surface morphology and topography of materials at very high magnification. SEMs can be used to study the crystalline structure of materials. By analyzing electron diffraction patterns, researchers can determine the crystallographic orientation and phase of samples. SEMs are particularly well-suited for surface analysis. They can provide detailed information about the surface topography, roughness, and texture of materials.

4.1.2.2 FTIR-Spectrometry

Fourier-transform infrared (FTIR) spectroscopy is a powerful analytical technique used to obtain information about the chemical composition and molecular structure of materials. FTIR spectroscopy is widely used for qualitative and quantitative analysis of organic and inorganic compounds. It can identify functional groups, detect impurities, and characterize chemical reactions. FTIR spectroscopy measures the absorption or emission of infrared light by molecules. Molecules absorb infrared radiation at specific frequencies

corresponding to the vibrational modes of their chemical bonds. By analysing the pattern of absorption, FTIR can provide information about the functional groups and molecular structure of a sample. 4.1.3 Preparation of Paramylon for Seed priming

Paramylon extracted from *Euglena gracilis* is taken and chemicals such as Sodium Hydroxide (0.5M) , Absolute Ethanol are collected along with distilled water. Three different concentrations of Paramylon (0.25mg/ml, 0.5mg/ml, 1mg/ml) are taken for the priming of seeds. Each concentration is dissolved in 1ml of 0.5M NaOH (Sodium Hydroxide) separately in Falcon tubes. After complete mixing, 2ml of Absolute Ethanol (99%) is added to the three tubes. Centrifuge the mixture at 7000rpm for 15 minutes at 4°C. After centrifugation, take the tubes and completely drain out the supernatant. Redissolve the pellet in 400 microlitres of distilled water. Again, add 800 microlitres of Absolute Ethanol. Mix well. Centrifuge for 15 minutes at 7000rpm at 4°C. Remove the supernatant and wash the pellet 2-3 times. Make the volume up to 5ml with distilled water

4.1.4 Seed priming

The Sample- Mung bean seeds are collected. Sodium Hypochlorite solution is taken. Mung bean seeds are surface sterilized with 2% Sodium hypochlorite for 10 minutes. The sterilized seeds are imbibed in water for 30 minutes. After 30 minutes, 10 to 20 seeds are randomly selected and incubated in the three different concentrations of Paramylon for 24 hours at room temperature. Another set of seeds is kept unprimed.

4.1.5 Preparation of Cadmium chloride (CdCl₂) for Cadmium stress condition

Stock solution	1M concentration of Cadmium chloride
Calcium chloride	1.8332 gram
Distilled water	10ml

□ Stock solution – 1.8332g of CdCl₂ is dissolved in 10ml of distilled water.

Working standard 1 0.5mM CdCl ₂	125µl from stock solution added to 250ml of distilled water
Working standard 2 1mM CdCl ₂	250µl from stock solution added to 250ml of distilled water

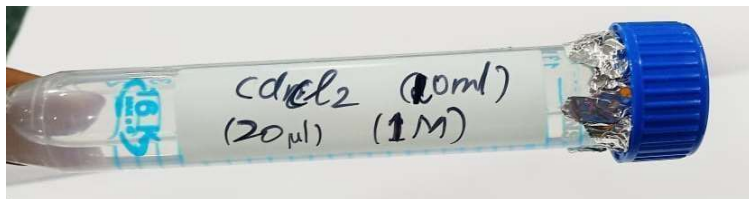


Fig: 4.2 Calcium chloride Stock solution



Fig: 4.3 Working standard 1&2 (0.5mM & 1mM CdCl₂ Stress condition)

4.1.6 Incubation of seeds for germination

After 24 hours, the primed seeds are taken out. 10 seeds are selected from each concentration of paramylon and are kept for germination in the germination sheet separately. Now the sheets are incubated inside the prepared working standard 1 and (0.5mM and 1mM Cadmium stress condition). 10 unprimed seeds are kept for Control.



Fig 4.4 Seedlings after 7 days of germination grown under 0.5mM Cd stress



Fig: 4.5 Seedling after 7 days of germination grown under 1mM Cd stress The growth of the seedlings is observed for 7 days. The root length, shoot length, and fresh weight are noted. The dry weight is calculated after drying the seedlings in a hot air oven for 48

hours at 80° Celsius. The germination rate is calculated according to (Akash Hidangmayum & Padmanabh Dwivedi, 2022). The seedling vigour index is also calculated accordingly.

$$\text{Germination \%} = (\text{Number of seeds germinated} / \text{Total Number of seeds}) \times 100$$

$$\text{Seedling Vigour Index} = \text{Shoot length(cm)} + \text{Root length (cm)} \times \text{Germination \%}$$

4.1.7 Total Chlorophyll content

The materials required for estimation of chlorophyll content are Acetone, Mortar and pestle, and falcon tubes. 0.1g of leaves are ground with 5ml of acetone. The extract is centrifuged at 4000 rpm for 20 minutes. Now the supernatant is collected and the residue is again re-extracted with 5ml of acetone and centrifuged at 4000rpm for 20 minutes. The supernatants are combined. The final volume is made to 10ml. Absorbance was measured at 645nm and 663nm for Chlorophyll a and Chlorophyll b respectively in a microplate reader.

Total Chlorophyll content = $(22.2 \times A_{645} + 8.02 \times A_{663}) \times V/W \times 1/1000$ where, A= absorbance of the chlorophyll extract at the specific wavelength.

V= final volume of the 80% acetone chlorophyll extract.

W= Fresh weight in grams of the tissue extracted.



Fig: 4.6 Centrifugation of leaf extract

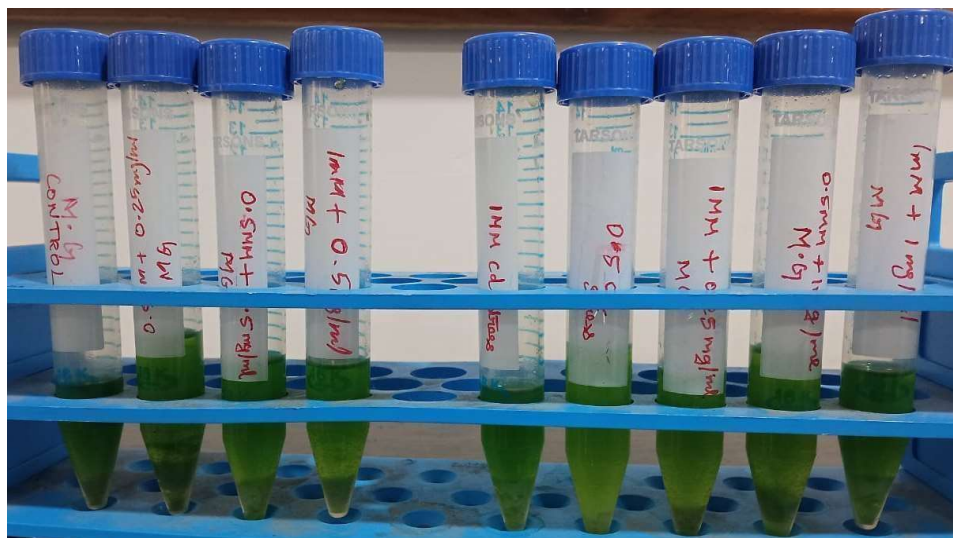


Fig: 4.7 Leaf extract for Estimation of Chlorophyll content

4.1.8 Determination of ROS accumulation in root tips

The required materials are surgical Blade, Tweezers, Eppendorf tubes, distilled water, 2',7' - Dichlorodihydrofluorecein diacetate (DCFH-DA) dye.

Root tips of Mung bean seedlings from each concentration are equally cut around 2 to 3 cm using a surgical blade. The root tips are washed in distilled water by suspending them in small Eppendorf tubes. Then, the root tips are taken out and dried gently. Now the root tips are stained with 25 μ M 2',7' -

Dichlorodihydrofluorecein diacetate (DCFH-DA) dye for 15 minutes (Ghosh et al., 2015). The root tips are observed under a Confocal Laser Microscope to determine the accumulation of ROS in the root tips of the seedlings.

4.1.9 Determination of Cadmium accumulation in root tips

Here, Leadmium green dye is used to determine the accumulation of Cadmium in the roots of *Vigna radiata*. Root tips of Mung bean seedlings from each concentration are equally cut around 2 to 3 cm using a surgical blade. The root tips are washed in distilled water by suspending them in small Eppendorf tubes. Then, the root tips are taken out gently and dried. Now the roots are stained with

Leadmium Green dye. The Root tips are observed under a Confocal Laser Microscope for qualitative analysis of the accumulation of cadmium in root tips.



Fig: 4.8 Cutting of root tips using a surgical blade



Fig: 4.9 Collection of root tips for staining

4.1.10 Estimation of Anti-oxidant enzymes

Fresh leaves (0.5g) were completely grounded in 5 ml of Potassium phosphate buffer (50 mM) under ice-cold conditions and centrifuged at 15,000 rpm and 4°C. The supernatant is collected and stocked at -20°C for determination of antioxidant activity (Khan et al., 2021).

4.1.10.1 Determination of Catalase enzyme activity

To 100 µl of leaf extract, 2.5 ml of potassium phosphate buffer and 100 µl of H₂O₂ are added and mixed gently. For determination of CAT activity, the solution is observed at 240 nm in a spectrophotometer. The extinction coefficient is taken as 40 mM⁻¹ cm⁻¹ (Khan et al., 2021).

4.1.10.2 Determination of Superoxide dismutase enzyme activity

SOD activity was assayed by measuring enzyme capability for photochemical reduction of nitro blue tetrazolium (NBT). The reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7.5), 1500 mM sodium carbonate, 200 mM methionine, 2.25 mM NBT, 3 mM EDTA, 1 mL distilled water and 150 µL of leaf extract incubated at dark for 8 min and 60 µM riboflavin was added at the end. Vortex the reaction mixture and place 30 cm below the light source consisting of 40 W fluorescent lamps for half an hour. Tubes kept in the dark were served as a blank, while the control tube was without the enzyme and kept in the light. The absorbance was measured at 560 nm (Hassan et al., 2021).

4.1.10.3 Determination of Ascorbate peroxidase enzyme activity

0.5mM of Ascorbic acid and 2mM of H₂O₂ are prepared initially. 2.5 ml of 0.5 mM ascorbic acid, 0.4 ml of 2 mM H₂O₂ and 0.1 ml of the leaf extract were mixed. Then the decrease in absorbance at 290 nm was recorded. The extinction coefficient is 2.8 mM⁻¹ cm⁻¹. APX activity is determined by the protocol of Khan et al., 2021.

CHAPTER – 5

RESULTS AND DISCUSSION

5.1 CHARACTERISATION OF PARAMYLON

5.1.1 SEM analysis

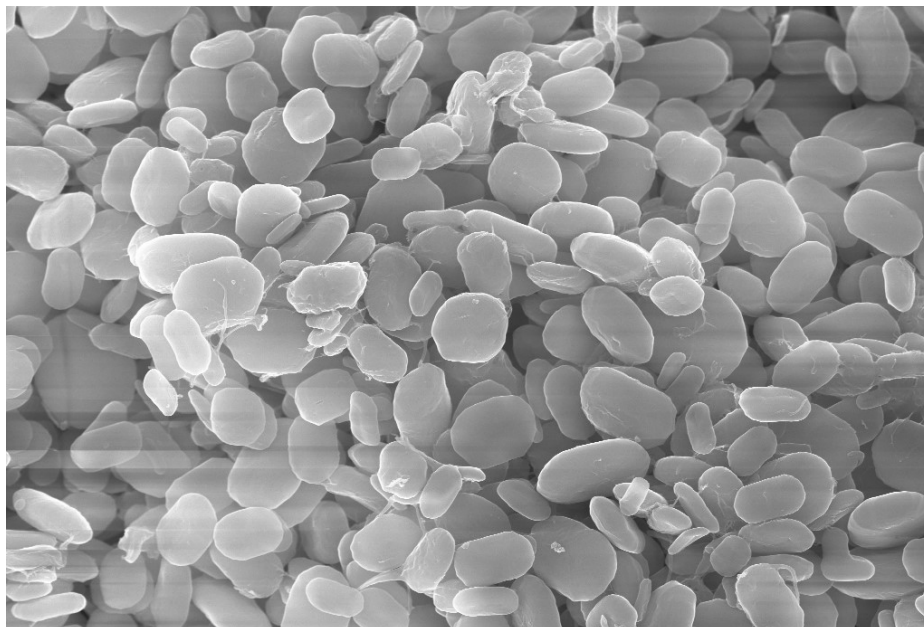


Fig: 5.1 Scanning Electron microscopic image of Paramylon

Paramylon granules extracted from the cell wall of *Euglena gracilis* were observed under Scanning Electron Microscopy. The resultant image revealed the morphological characteristics of the granules. They appear as clusters of granules in an oval, slightly elongated shape. Paramylon granules exhibit high electron density, so they appear as bright regions in the SEM image against a darker background. The surface of the granules appears to be smooth. However, the appearance of the paramylon may vary depending on factors such as the species of algae, the growth conditions and the sample preparation techniques. The present image agrees with the Liwei et al., 2022 characterization of Paramylon before H_2O_2 degradation. This crude form of paramylon is efficiently used for the seed priming process.

5.1.2 FTIR- Spectroscopy analysis

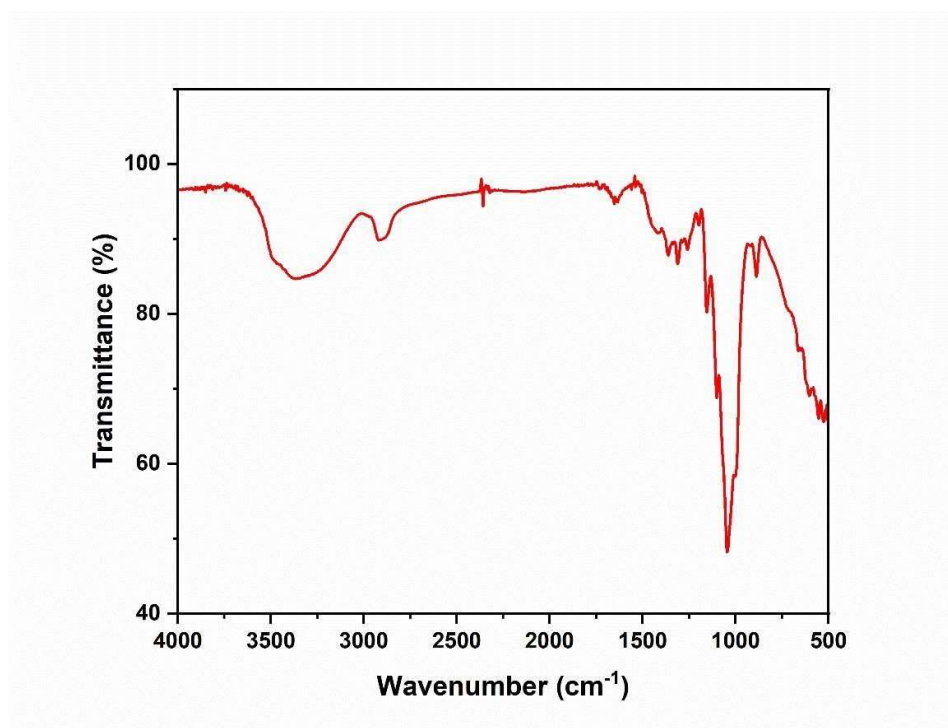


Fig: 5.2 FTIR Spectra of paramylon

Paramylon primarily consists of a β -1,3-glucan backbone, which shows characteristic peaks in the FTIR spectra. The β -glycosidic linkage between glucose units typically results in strong absorptions around 890-900 cm^{-1} . Glucose monomers in paramylon contain hydroxyl groups (-OH), which contribute to absorption bands in the region of 3200-3500 cm^{-1} (stretching vibrations) and around 1000-1200 cm^{-1} (bending vibrations). The intensity and position of these bands can provide information about the hydrogen bonding and hydration state of the paramylon. The stretching vibrations of C-H bonds can be observed in the region of 2800-3000 cm^{-1} . There is a peak at 1020 cm^{-1} which indicates a strong bond of C-OH. Vibrations near 1480 cm^{-1} to 1440 cm^{-1} indicate CH₂ bend at a medium level. Liwei et al., 2022, observed the same kind of absorbance in the untreated Paramylon.



Fig: 5.3 Mung bean seedlings treated with different concentrations of Paramylon grown under 0.5mM Cd stress



Fig: 5.4 Mung bean seedlings treated with different concentrations of Paramylon grown under 1mM Cd stress

5.2 GROWTH CHARACTERISTICS OF MUNG BEAN SEEDLINGS

5.2.1 Root length of Mung bean seedlings

Table: 5.1 Root length (in cm) – 0.5mM Cd stress + Paramylon priming

Treatments	Control	0.5mM Cd stress	0.5mM Cd stress + 0.25P	0.5mM Cd stress + 0.5P	0.5mM Cd stress + 1P
Root Length	16.5cm	6cm	11.5cm	12.8cm	13.6cm

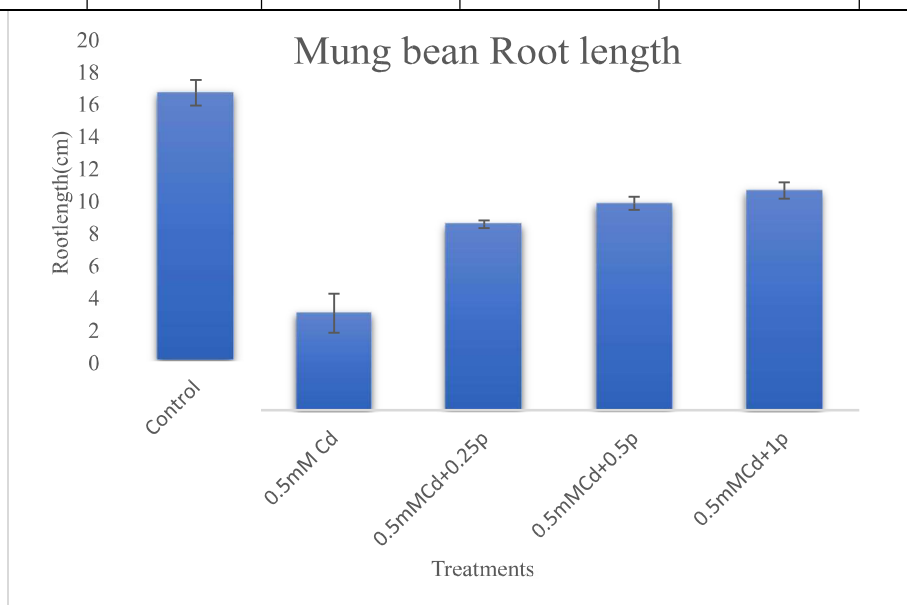


Fig: 5.5 Comparison between Root lengths of Mung bean seedlings (0.5mM Cd stress) coated with different concentrations of Paramylon

From the observed growth of the roots, the length of the roots has been drastically reduced when only 0.5mM of stress is applied to the Mung bean (*Vigna radiata*) seeds. Meanwhile, when the seeds are primed with Paramylon there is a gradual increase in the length of the roots which gives us a positive result from seedpriming. Under 0.5mM Cadmium stress condition, primed seeds of concentration 0.25mg/ml show gradual recovery of seeds from the stress effect. At, 1mg/ml concentration of priming, the seeds seem to grow without much effect of the stress.

Table: 5.2 Root length (in cm) – 1mM Cd stress+ Paramylon priming

Treatments	Control	1mM Cd stress	1mM Cd stress + 0.25P	1mM Cd stress + 0.5P	1mM Cd Stress + 1P
Root length (in cm)	16.5	6.1	14.3	14.7	15.4

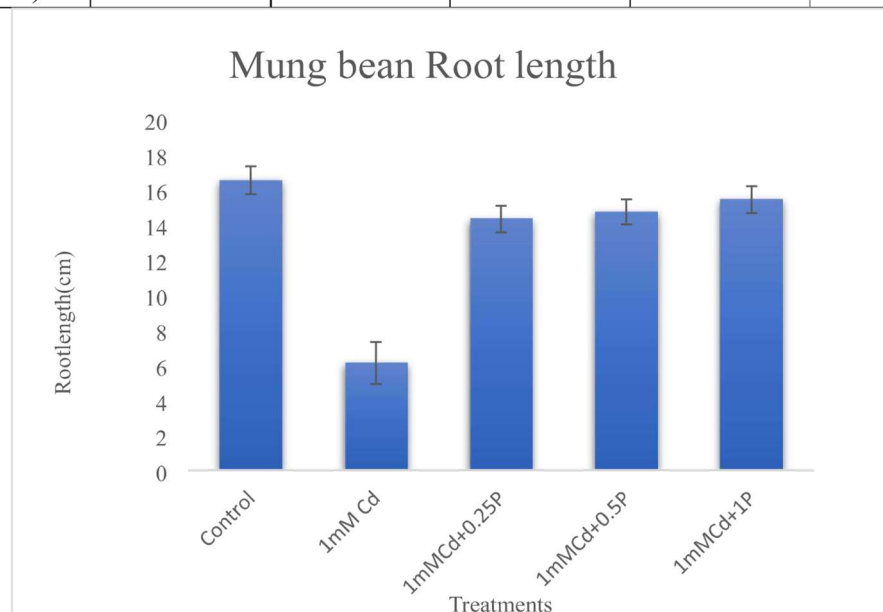


Fig: 5.6 Comparison between Root lengths of Mung bean seedlings (1mM Cd stress) coated with different concentrations of Paramylon

The root growth has been reduced nearly by 20% compared to Control under 1mM Cd stress level. When Paramylon is coated on the seeds, the root lengths of the seedlings are not reduced to an extent resulting in the recovery of the seedlings from the stress condition. At a higher concentration of coating, that is, 1mg/ml of Paramylon, the length of the roots is nearly equal to that of the Control. Also, the investigation done by Khan et al., 2021 observed increased in root growth after priming salicylic acid against cadmium stress on mung bean seed

5.2.2 Shoot Length of the Mung bean seedlings:

Table: 5.3 Shoot length (in cm) – 0.5mM Cd stress + Paramylon priming

Treatments	Control	0.5mM Cd stress	0.5mM Cd stress + 0.25P	0.5mM Cd stress + 0.5P	0.5mM Cd stress + 1P
Shoot Length (in cm)	15.5	9	11.1	14	18

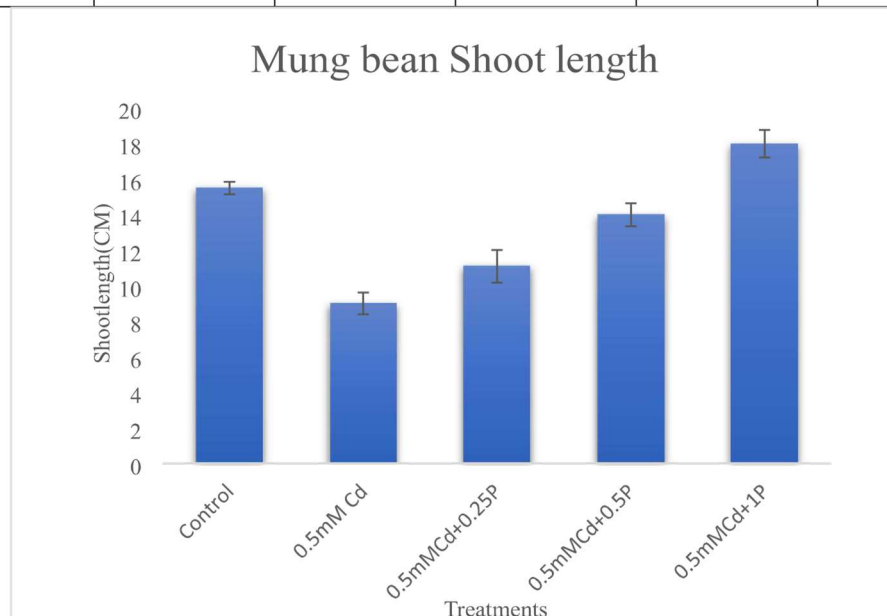


Fig: 5.7 Comparison between shoot length of Mung bean seedlings (0.5M Cd stress) coated with different concentrations of Paramylon

Cadmium stress (0.5mM) reduced the shoot growth by 50%. Meanwhile, the seeds primed with Paramylon show drastic growth in the shoot length. The shoot growth is more compared to the control (unprimed and without stress condition). Therefore, the shoot length of the seedlings is enhanced by the priming of Paramylon on the seeds of *Vigna radiata*. The shoot length is directly proportional to the concentration of paramylon used for priming.

Table: 5.4 Shoot length (in cm) – 1mM Cd stress + Paramylon priming

Treatments	Control	1mM Cd stress	1mM Cd stress + 0.25P	1mM Cd stress + 0.5P	1mM Cd Stress + 1P
Shoot Length (in cm)	15.5	9	12.7	14.5	16

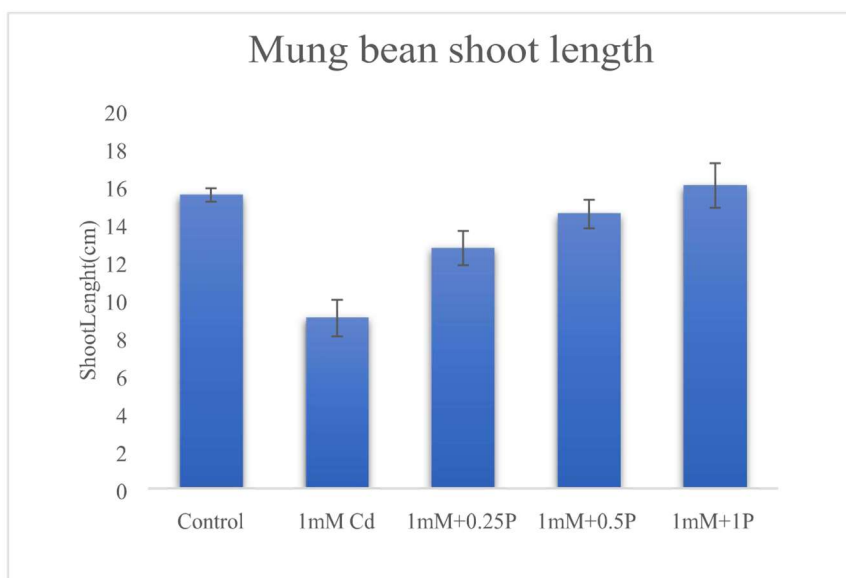


Fig: 5.8 Comparison between Root lengths of Mung bean seedlings (1mM Cd stress) coated with different concentrations of Paramylon

In the 1mM Cd stress condition, the shoot length was reduced to half. While the seeds are primed with paramylon the stress effect is completely neglected and the paramylon coating enhances the shoot growth which directly supports the seedling growth. Thus, paramylon has a positive effect on both the shoot and root lengths of mung bean seedlings under cadmium stress. Priming of mung bean with Phytohormones and proline against cadmium toxicity also improved the root and shoot lengths of the seedlings (Hassan et al., 2021).

5.2.3 Fresh weight of the Mung bean seedlings

Table: 5.5 Fresh weight (in grams) – 0.5mM Cd stress + Paramylon priming

Treatment	Control (in mg)	0.5mM Cd	0.5mMCd+ 0.25P	0.5mMCd+ 0.5P	0.5mMCd+ 1P
Fresh weight (mg)	175.9	116.8	161.2	172.5	182.5

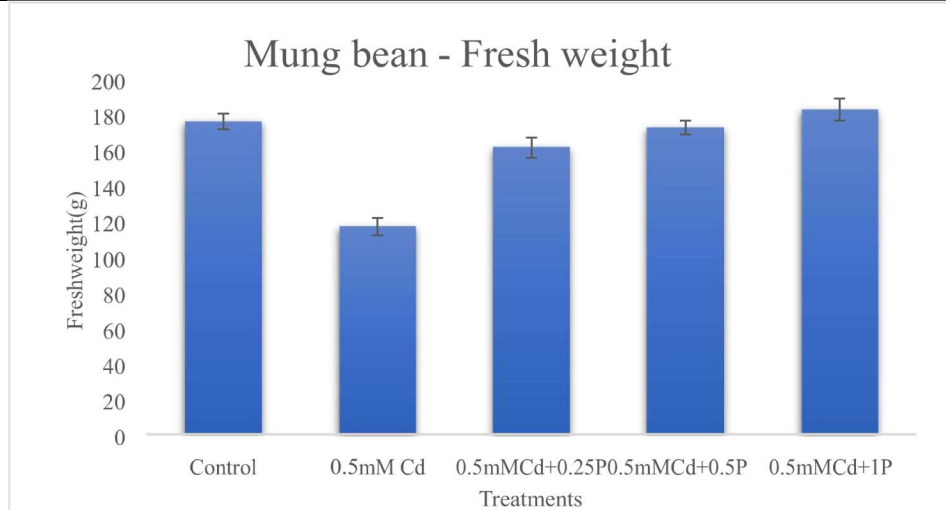


Fig: 5.9 Fresh weight(g) comparison of the Mung bean seedling coated with different concentrations of Paramylon under 0.5mM Cd stress

Fresh weight is the overall mass of the fresh seedlings taken after an incubation period of seven days. The Cd stress level – 0.5mM reduced the fresh weight of the seedlings to a notable extent, whereas the Paramylon primed seeds showed an increase in the fresh weight compared to the control and stress-affected seedlings. Priming showed that seeds can overcome the effect of cadmium toxicity and also helped the seedlings to have a gradual increase in fresh weight. The concentration of 1mg/ml paramylon priming is effective compared to 0.25mg/ml and 0.5mg/ml concentrations in overcoming the stress. Similar results have been observed in research done by Sharma et al., 2021.

Table: 5.6 Fresh weight (in grams) – 1mM Cd stress + Paramylon priming

Treatments	Control	1mM Cd	1mMCd+ 0.25P	1mMCd+ 0.5P	1mMCd+ 1P
Fresh weight (mg)	175.9	111	130.8	150.4	208.3

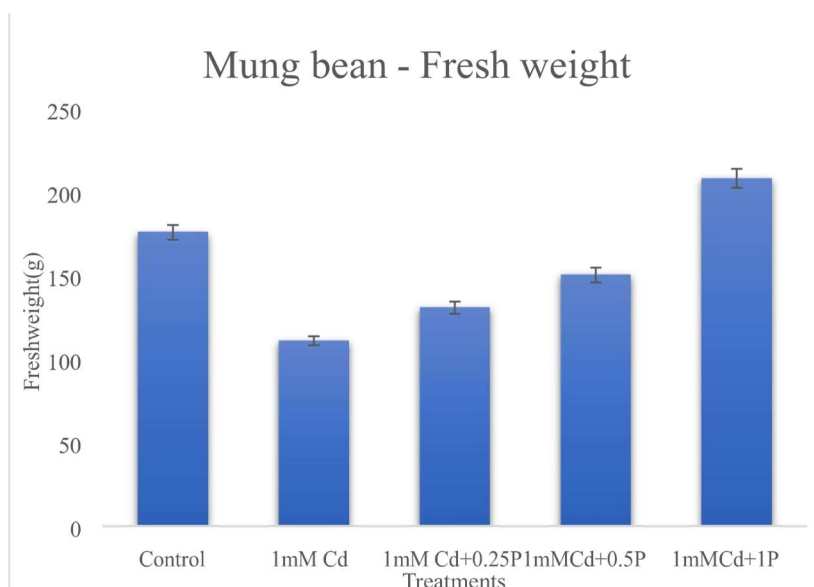


Fig: 5.10 Fresh weight(g) comparison of the Mung bean seedling coated with different concentrations of Paramylon under 1mM Cd stress

Fresh weight is the overall mass of the fresh seedlings taken after an incubation period of seven days. The Cd stress level – 1mM reduced the fresh weight of the seedlings to an extent, whereas the Paramylon primed seeds showed an increase in the fresh weight compared to the control and stress-affected seedlings. Priming showed that seeds can overcome the effect of cadmium toxicity and also helped the seedlings to have a gradual increase in fresh weight. The concentration of 1mg/ml paramylon priming is the most effective such that the weight is increased by 53% than the stress-induced unprimed seedlings.

5.2.4 Dry weight of the Mung bean seedlings

Table: 5.7 Dry weight (in grams) – 0.5mM Cd stress + Primed seedlings

Treatments	Control	0.5mM Cd	0.5mM Cd+ 0.25P	0.5mM Cd+ 0.5P	0.5mM Cd+ 1P
Dry weight (mg)	17.2	10.2	14.2	16.5	20

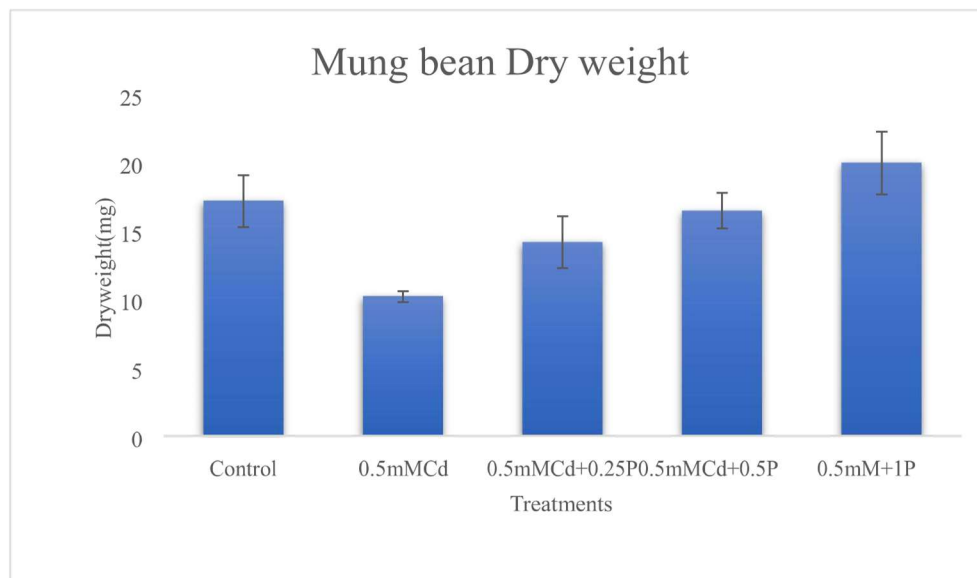


Fig: 5.11 Dry weight(g) comparison of the Mung bean seedling coated with different concentrations of Paramylon under 0.5mM Cd stress

There is a peak in the dry weight of the seedlings at 1mg/ml Paramylon primed seeds under 0.5mM Cd stress level. This dry weight is comparatively more than the Control. The seedlings that are grown after priming high concentrations of paramylon do not lose their dry weight even under cadmium stress.

Table: 5.8 Dry weight (in milligrams) – 1mM Cd stress + Primed seedlings

Treatments	Control	1mM Cd	1mMCd+ 0.25P	1mMCd+ 0.5P	1mMCd+ 1P
Dry weight (mg)	17.72	11.6	15	18.1	23.9

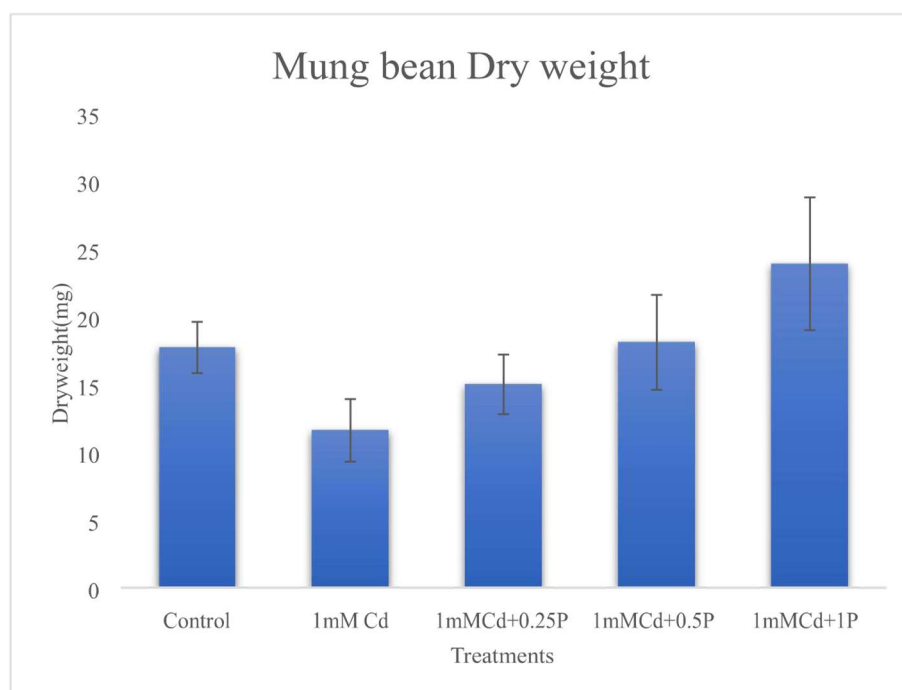


Fig: 5.12 Dry weight(g) comparison of the Mung bean seedling coated with different concentrations of Paramylon under 1mM Cd stress

The dry weight of the seedlings grown under 1mM cd stress condition is likely to have the least value of dry weight compared to other treatments. Meanwhile, the primed seedlings showed an increase in dry weight values even under stress conditions of 1mM. Thus, priming with a high concentration of paramylon – 1mg/ml can effectively influence the dry weight of the plant.

5.2.5 Germination Percentage of the Mung Bean Seedlings

Table: 5.9 Germination % – 0.5mM Cd stress + Primed seedlings

Treatments	Control	0.5mM Cd	0.5mMCd+ 0.25p	0.5mMCd+ 0.5p	0.5mMCd+ 1p
Percentage	100%	40%	50%	55%	85%

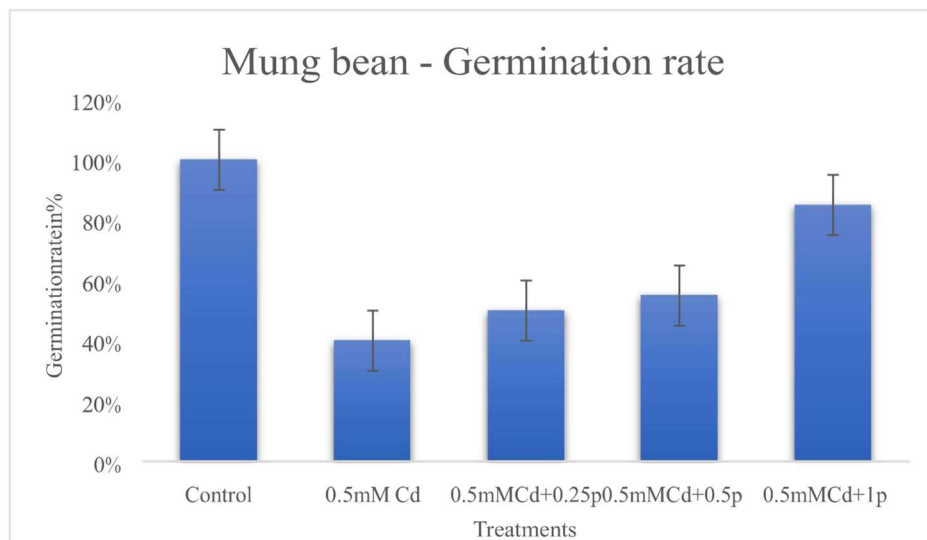


Fig: 5.13 Germination rate of the seedlings primed with different concentrations of Paramylon and grown under 0.5mM Cd stress

Germination percentage is the rate at which a seed grows daily. Germination percentage is an important trait in calculating the yield of a plant. The Control seedlings, that is, seeds grown under normal conditions without inducing stress and without priming have a 100% germination rate. When cadmium stress is induced at 0.5mM level the germination rate is 40%. The seeds which are primed with paramylon and grown under 0.5mM stress condition showed an increase in the stress condition as the concentration of paramylon coating increased. 0.25mg/ml paramylon priming had a germination rate of 50% and 0.5mg/ml paramylon priming had a germination rate of 55% and the germination rate is higher in seeds that are coated with paramylon at the concentration of 1mg/ml.

Table: 5.10 Germination % – 1mM Cd stress + Primed seedlings

Treatments	Control	1mM Cd	1mM Cd+ 0.25P	1mM Cd+ 0.5P	1mM Cd+ 1P
Percentage	100%	35%	60%	65%	90%

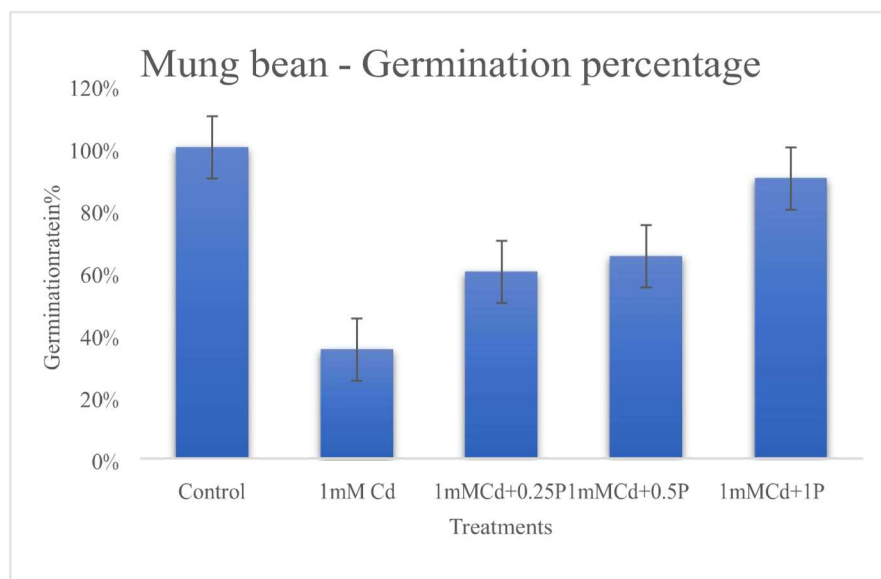


Fig: 5.14 Germination rate of the seedlings primed with different concentrations of Paramylon and grown under 1mM Cd stress

The daily rate of growth of a seed is its germination percentage. A key characteristic in determining a plant's yield is its germination percentage. With no stress and no priming, the control seedlings—seeds produced under regular conditions—have a germination rate of 100%. There is a 35% germination rate when 0.5 mM cadmium stress is introduced. The stress condition increased with an increase in the paramylon coating concentration in seeds primed with paramylon and grew under 0.5mM stress conditions.

5.2.6 Seedling Vigour Index

Table: 5.11 Seedling Vigour Index – 0.5mM Cd stress + Primed seedlings

Treatments	Control	0.5mM Cd	0.5mM Cd+ 0.25p	0.5mM Cd+ 0.5p	0.5mM Cd+ 1p
Value	32	9.6	19.15	14.025	26.18

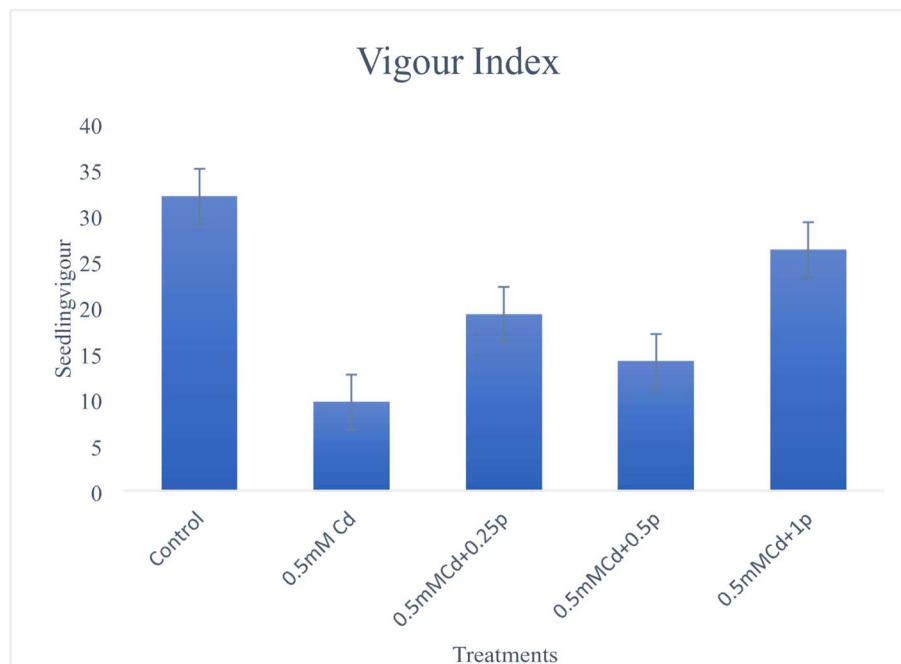


Fig: 5.15 Vigour Index of the Seedlings under 0.5mM Cd stress treated with various concentrations of Paramylon

Seedling vigour refers to seedlings' overall health, vigour, and robustness during the early stages of plant growth. It encompasses various aspects of seedling performance, including germination rate, emergence speed, root and shoot growth, and resistance to biotic and abiotic stresses. Seedling vigour is comparatively reduced even in the primed seeds than in the control. However, the seed has overcome the stress condition induced after priming the seeds with paramylon. The Vigour index of non-primed seeds is reduced to 20% in 0.5mM cd stress level meanwhile, paramylon primed seeds have an 80% vigour index.

Table: 5.12 Seedling Vigour Index – 1mM Cd stress + Primed seedlings

Treatments	Control	1mM Cd	1mM Cd+ 0.25P	1mM Cd+ 0.5P	1mM Cd+ 1P
Value	32	9.5	16.2	18.98	28.26

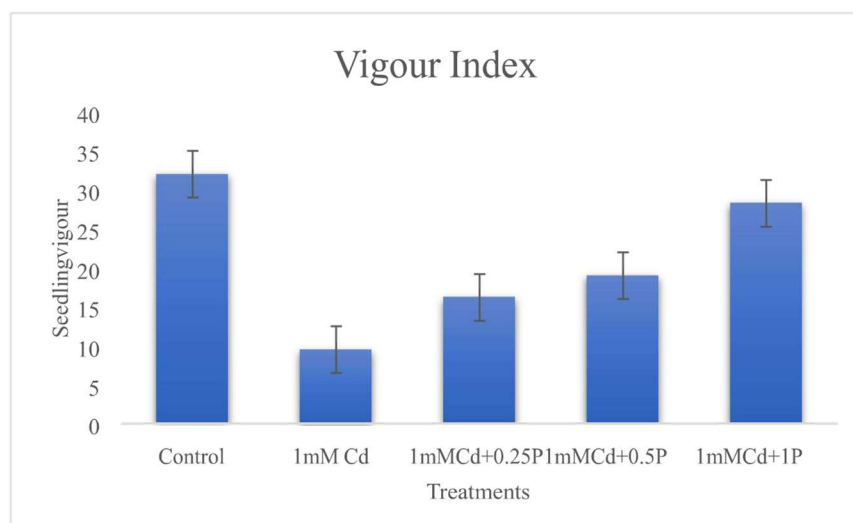


Fig: 5.16 Vigour Index of the Seedlings under 1mM Cd stress treated with various concentrations of Paramylon

The Vigour Index is inhibited for the seedlings under a 1mM stress level. Seeds primed with a high concentration of paramylon (1mg/ml) show a higher vigour index compared to seeds primed with 0.5mg/ml and 0.25mg/ml concentrations. However, the vigour index is slightly low compared to the control. Thus, the seedling vigour index can be enhanced even in the cadmium toxic condition if *Vigna radiata* seeds are coated with Beta-1,3-glucan (Paramylon).

5.3 TOTAL CHLOROPHYLL CONTENT

Table: 5.13 Chlorophyll content present in leaves of Mung bean seedlings treated under 0.5mM Cd stress

Treatments	Control	0.5mM Cd	0.5mMCd+0.25p	0.5mMCd+0.5p	0.5mMCd+1p
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Value	0.678	0.6114	0.621	0.635	0.664
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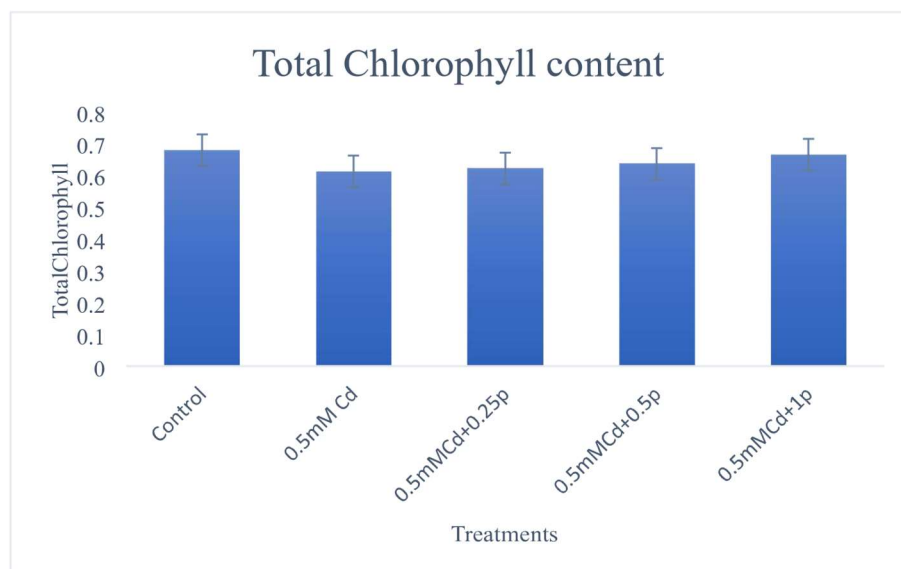


Fig: 5.17 Total chlorophyll content from the leaf extract of the Mung bean seedlings (0.5M Cd stress) treated with various concentrations of Paramylon

Chlorophyll in leaves is determined for several reasons, primarily because it serves as a key indicator of plant health, photosynthetic capacity and stress responses. Stressinduced chlorophyll degradation or inhibition of chlorophyll synthesis pathways can result in pale or yellowish leaves. Cadmium stress affects the chlorophyll content in the leaves to the maximum extent. The control has high chlorophyll content while the 0.5mM level of cadmium stress has the lowest chlorophyll content. Seedlings primed with paramylon show a gradual increase in the chlorophyll value as the concentration of the paramylon also increases. The same trend is observed in (Akash Hidangmayum & Padmanabh Dwivedi, 2022).

Table: 5.14 Chlorophyll content present in leaves of Mung bean seedlings treated under 1mM Cd stress

Treatments	Control	1mM Cd stress	1mM Cd stress + 0.25P	1mM Cd stress + 0.5P	1mM Cd Stress + 1P
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Value	0.678	0.585	0.623	0.69	0.724
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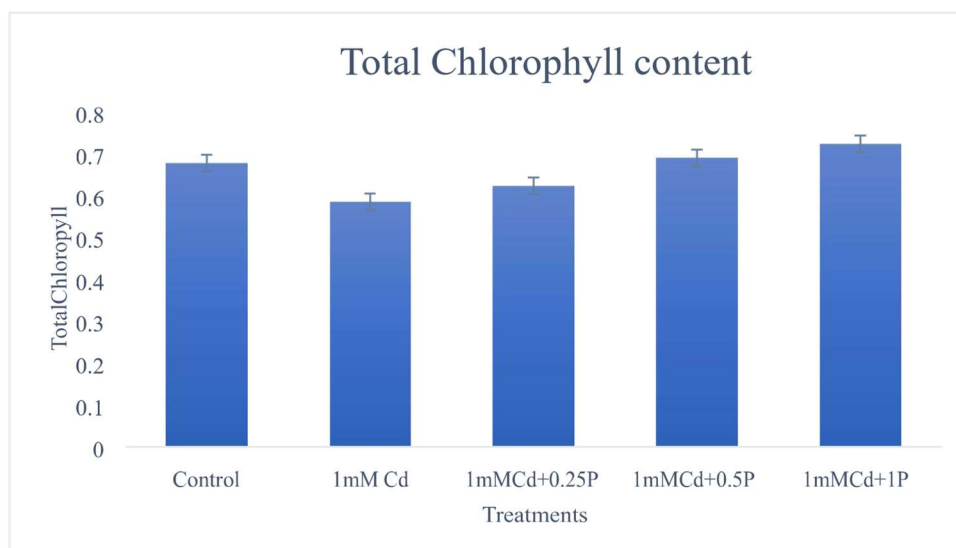
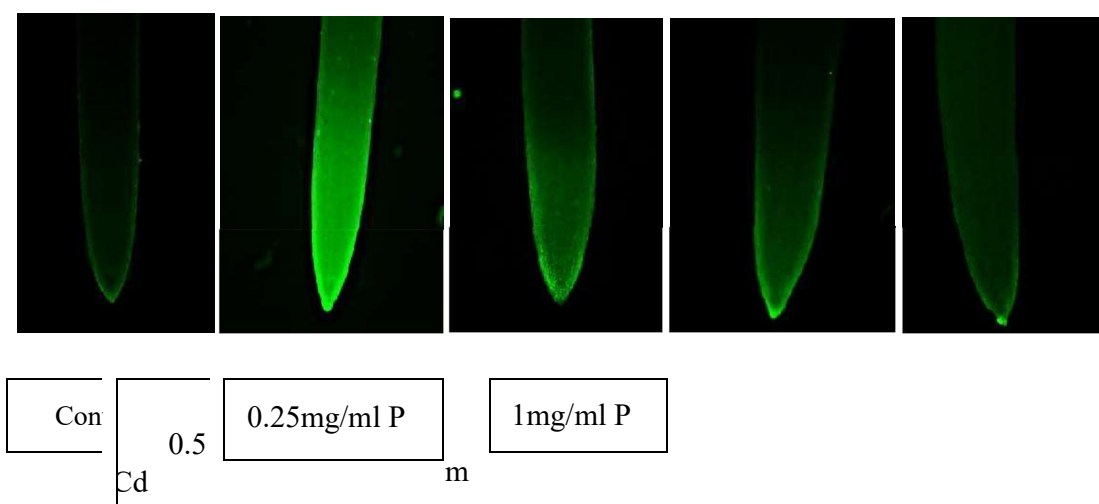


Fig: 5.18 Total chlorophyll content from the leaf extract of the Mung bean seedlings (1mM Cd stress) treated with various concentrations of Paramylon

There is only a slight difference in the total chlorophyll content in the mung bean seedlings. However, the stress-induced seedlings without priming of paramylon have the lowest value of chlorophyll content. Also, the primed seedlings with 1mg/ml concentration of paramylon have higher chlorophyll content than the control. The total chlorophyll content is increased by priming the seeds with paramylon under cadmium stress. The paramylon neglects the toxic effect on the seed and helps the seedling to grow in a healthier way.

5.4 ROS ACCUMULATION IN ROOT TIPS OF THE SEEDLINGS



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Fig: 5.19 Images depicting ROS accumulation in root tips of Mung bean seedlings grown under 0.5mM Cd stress

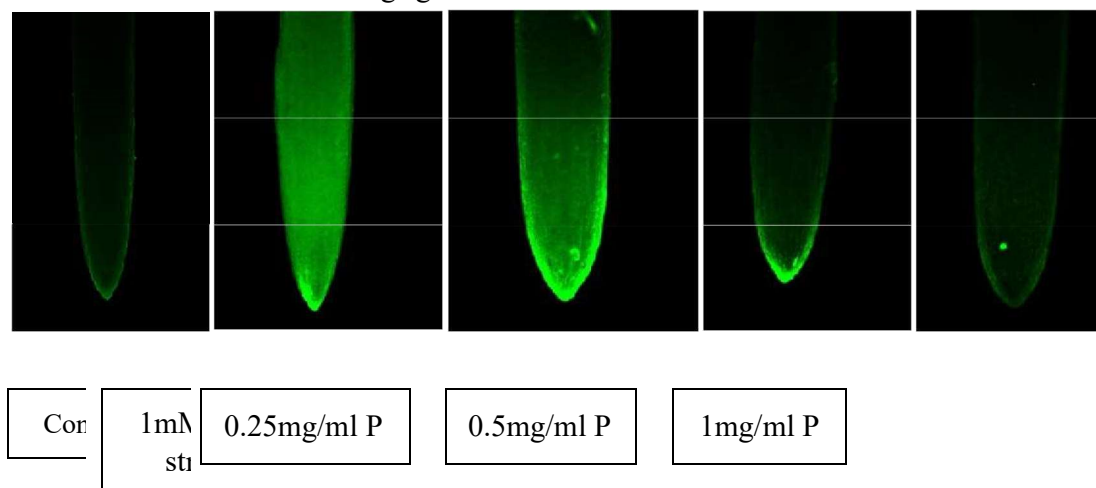


Fig: 5.20 Images depicting ROS accumulation in root tips of Mung bean seedlings grown under 1mM Cd stress

The bright fluorescence green in the root tips indicates the level of ROS accumulation in the tips. Unprimed seedlings grown under stress conditions show high level of ROS accumulation due to the induced stress damage in the roots of those seedlings. The root tips of seedlings primed with 1mg/ml paramylon concentration have no ROS accumulation.

The other two concentrations (0.5 mg/ml P & 1mg/ml P) have a low level of ROS accumulation in the edges of root tips which shows us that the seedlings have struggled against the toxicity of Cadmium.

5.5 ACCUMULATION OF CADMIUM IN ROOT TIPS OF THE SEEDLINGS

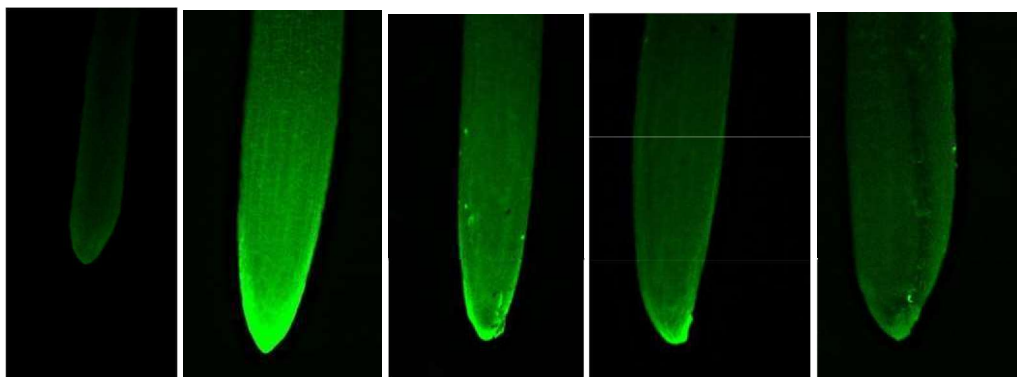


Fig: 5.21 Images depicting cadmium accumulation in root tips of Mung bean seedlings grown under 0.5mM Cd stress

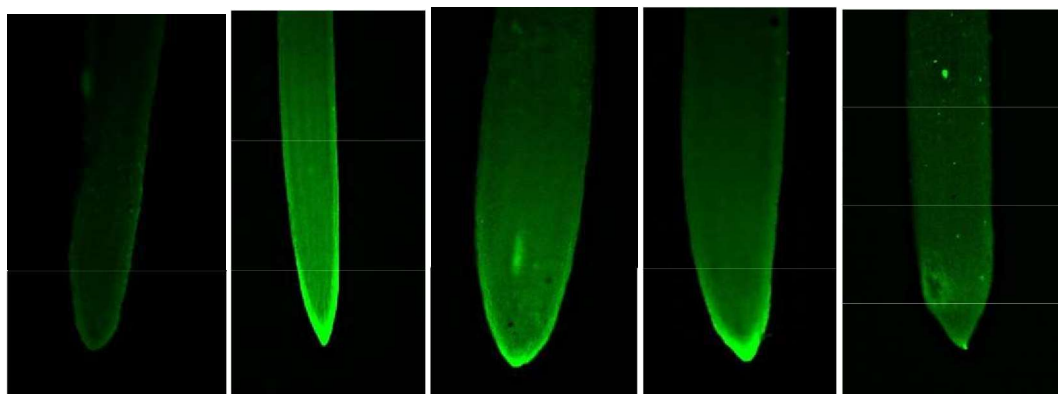


Fig: 5.22 Images depicting cadmium accumulation in root tips of Mung bean seedlings grown under 1mM Cd stress

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

that ultimately helps in the growth of the plant without any toxic effects from the cadmium stress.

5.6 DETERMINATION OF ANTIOXIDANT ENZYME ACTIVITY

5.6.1 Catalase Enzyme activity

Table: 5.15 Absorbance value of Catalase enzyme from plant extract of Mung bean seedlings grown in 0.5mM Cd stress

Treatment	Control	0.5mM Cd	0.5mMCd+ 0.25p	0.5mMCd+ 0.5p	0.5mMCd+ 1p
Absorbance value	0.55	0.26	0.34	0.44	0.58

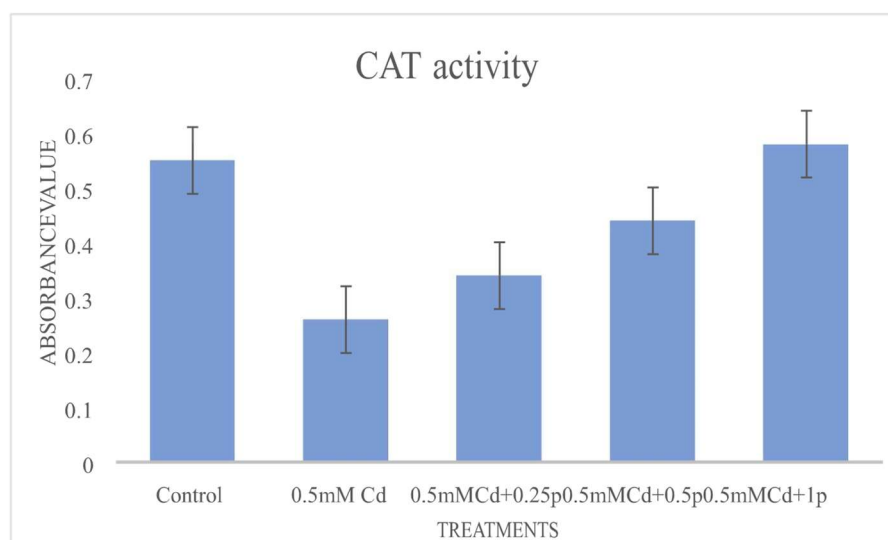


Fig: 5.23 Variations in CAT activity for the seedlings treated with various concentrations of Paramylon grown under 0.5mM Cd stress

Catalase is an antioxidant enzyme that acts as a catalyst for the conversion of hydrogen peroxide to oxygen and water. The above graph depicts the activity Catalase in the Mung bean seedlings. Catalase activity determines how plants resist to the applied stress. It is observed that the CAT activity was high in the seedlings that are primed with

high concentration of Paramylon (1mg/ml). The enzyme activity is inhibited in the unprimed seedlings which were grown under cadmium stress of 0.5mM.

Table: 5.16 Absorbance value of Catalase enzyme from plant extract of Mung bean seedlings grown in 1mM Cd stress

Treatments	Control	1mM Cd	1mMCd+ 0.25P	1mMCd+ 0.5P	1mMCd+ 1P
Absorbance value	0.55	0.29	0.38	0.46	0.59

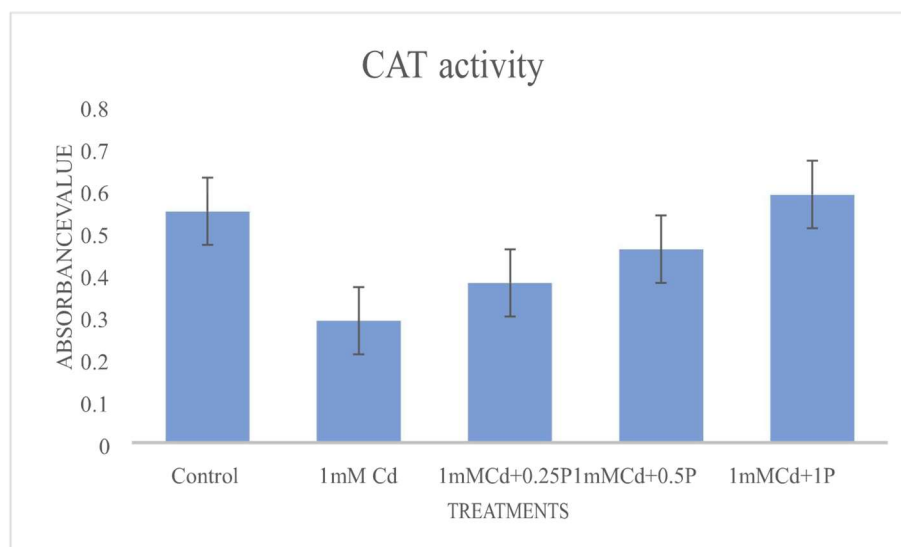


Fig: 5.24 Variations in CAT activity for the seedlings treated with various concentrations of Paramylon grown under 1mM Cd stress

CAT enzyme is responsible for neutralization through decomposition of hydrogen peroxide, thereby maintaining an optimum level of the molecule in the cell which is also essential for cellular signalling processes. The CAT activity is observed to be high in the seedlings primed with 1mg/ml concentration of Paramylon compared to the control. When the mung bean seedlings are grown only under 1mM Cd stress without the Paramylon priming, the Cat activity is inhibited to the maximum. A gradual increase in the Catalase enzyme activity is as the priming concentration of the Paramylon also increased.

5.6.2 Superoxide Dismutase enzyme activity

Table: 5.17 Absorbance value of Superoxide dismutase from plant extract of Mung bean seedlings grown in 0.5mM Cd stress

Treatments	Control	0.5mM Cd	0.5mMCd+ 0.25p	0.5mMCd+ 0.5p	0.5mMCd+ 1p
Absorbance value	0.46	0.29	0.35	0.42	0.48

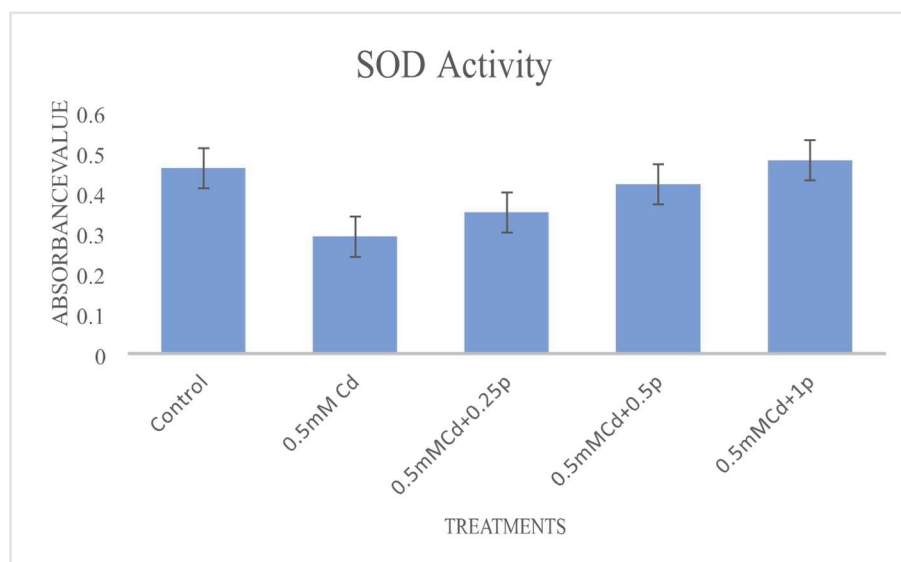


Fig: 5.25 Variations in SOD activity for the seedlings treated with various concentrations of Paramylon grown under 0.5mM Cd stress

In plant cells, superoxide dismutase (SOD) functions as a defense mechanism against reactive oxygen species (ROS). Superoxide radicals are dismutated into hydrogen peroxide (H₂O₂) and molecular oxygen (O₂) by SOD enzymes. When exposed to 0.5 mM of cadmium stress, SOD activity is 50% less than that of the control. In the meantime, when seeds are primed with a concentration of 1 mg/ml of Paramylon and cultivated under the same stress conditions, the enzyme activity increases by twice as much.

Table: 5.18 Absorbance value of Superoxide dismutase from plant extract of Mung bean seedlings grown in 1mM Cd stress

Treatments	Control	1mM Cd	1mMCd+0.25P	1mMCd+0.5P	1mMCd+1P
Absorbance value	0.46	0.21	0.33	0.39	0.47

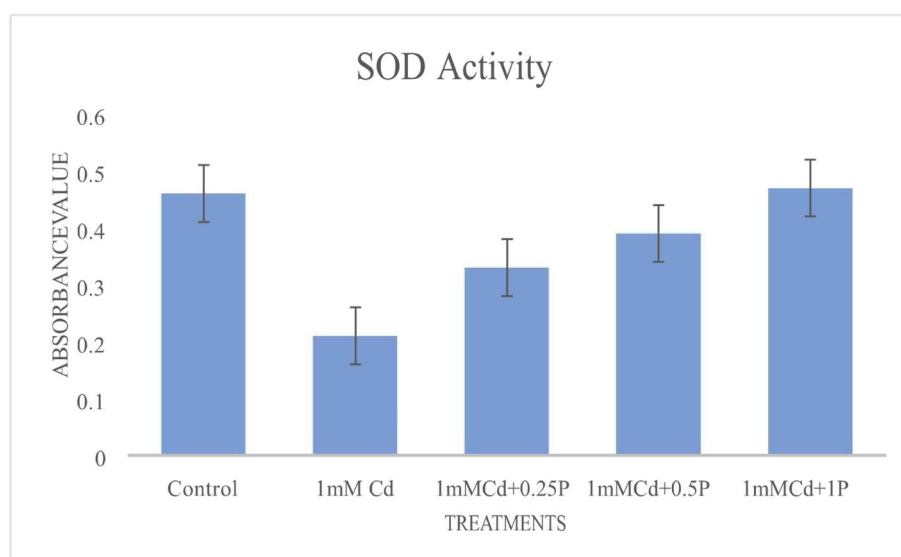


Fig: 5.26 Variations in SOD activity for the seedlings treated with various concentrations of Paramylon grown under 1mM Cd stress

The above graph demonstrates the activity of SOD in mung bean seedlings grown under 1mM Cd stress. The enzyme activity seems to be increasing after the paramylon when the seeds are primed with paramylon at different concentrations. The SOD activity was high in mung bean seedlings when primed with a high concentration of Paramylon even under 1mM cadmium stress level. Whereas when the seeds are unprimed and grown under the same stress level, the enzyme activity is reduced to half.

5.4.3 Ascorbate peroxidase enzyme activity:

Table: 5.19 Absorbance value of Ascorbate peroxidase from plant extract of Mung bean seedlings grown in 0.5mM Cd stress

Treatments	Control	0.5mM Cd	0.5mMCd+ 0.25p	0.5mMCd+ 0.5p	0.5mMCd+ 1p
Absorbance value	0.35	0.2	0.25	0.27	0.34

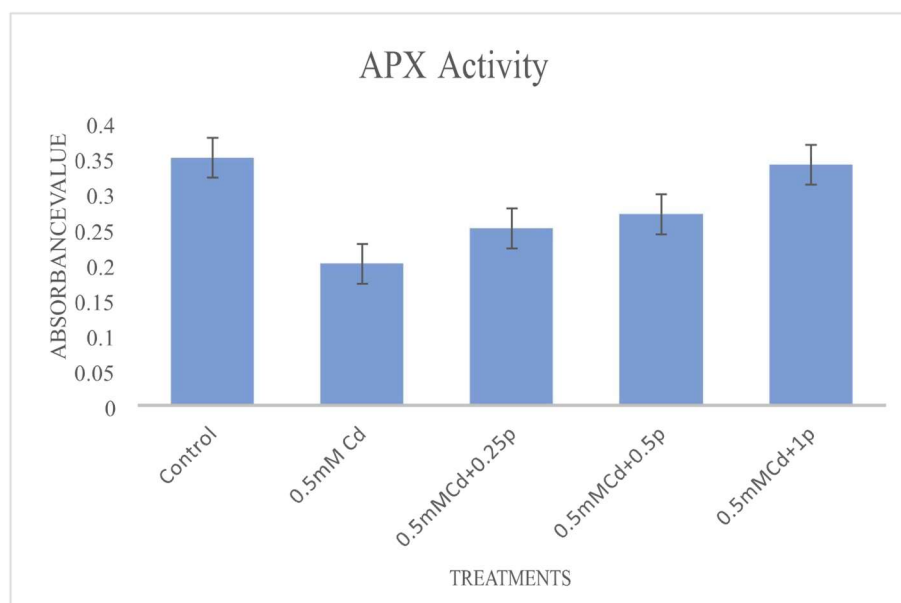


Fig: 5.27 Variations in APX activity for the seedlings treated with various concentrations of Paramylon grown under 0.5mM Cd stress

Ascorbate peroxidase (APX) is a key enzyme in the ascorbate-glutathione cycle, which is a major hydrogen peroxide detoxifying system in plant cells. A high level of APX activity in the plant cells indicate that the plant shows maximum resistant to the prevailing stress condition. The absorbance value is high in seedlings that are primed with 1mg/ml paramylon which in turn represent the high level activity of APX enzyme. The unprimed seed grown under 0.5mM stress condition show low absorbance value, hence the APX activity is low when the seeds are unprimed.

Table: 5.20 Absorbance value of Ascorbate peroxidase from plant extract of Mung bean seedlings grown in 1mM Cd stress

Treatments	Control	1mM Cd	1mMCd+ 0.25P	1mMCd+ 0.5P	1mMCd+ 1P
Absorbance value	0.35	0.18	0.22	0.27	0.33

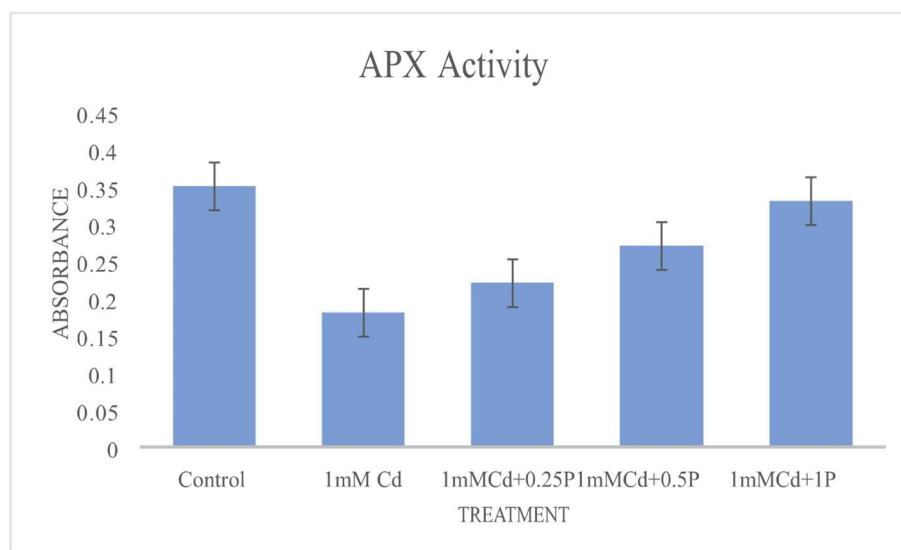


Fig: 5.28 Variations in APX activity for the seedlings treated with various concentrations of Paramylon grown under 1mM Cd

From the graph, we can infer that the APX activity is high in the control and it is 60% inhibited when 1mM of cadmium stress is applied to the seeds. When the seeds are primed with Paramylon at different concentrations the activity of APX is increased gradually. The high activity of the APX enzyme is displayed in the seedlings that are primed with 1mg/ml and 0.5 mg/ml of Paramylon. Therefore, priming method of Paramylon on mung bean seeds resulted in the enhancement of enzyme activities neglecting the cadmium stress condition.

CHAPTER 6

SUMMARY AND CONCLUSION

Seed priming of Mung bean seeds with Paramylon has a greater effect in overcoming cadmium toxicity to a greater extent. All the morphological traits such as root and shoot lengths, fresh and dry weight, germination rate and seedling vigour have been increased while the seeds are coated with Paramylon. Cadmium stress lead to toxicity symptoms in seedlings, including stunted growth, chlorosis, and reduced biomass accumulation. Seed priming treatments may mitigate these toxic effects by enhancing the detoxification mechanisms within seeds and seedlings, thereby promoting healthier seedling development. The total chlorophyll content of the seedlings also increased compared to the seedlings that are grown only under cadmium stress unprimed of Paramylon. The staining of DCFH - DA dye determined the ROS accumulation in the root tips which also confirms that the increase in paramylon priming in seeds decreases the ROS accumulation in the root tips. The staining procedure of Leadmium green dye used to determine the cadmium accumulation also proves that the seed priming effect of Paramylon will prevent the accumulation of cadmium in the root tips. The activity of antioxidants - Catalase, Superoxide dismutase and Ascorbate peroxidase is high in seedlings treated with 1mg/ml concentration of Paramylon which indicated that the plants resist Cadmium toxicity. Hence, from the overall study, we can conclude that priming of Paramylon on Mung bean (*Vigna radiata*) seeds can successfully overcome the cadmium effect on seedlings and germination and result in higher yielding capacity. Seed priming with paramylon has been shown to have positive effects on various aspects of seed germination and seedling growth in mung bean seeds. Paramylon priming has proved to improve the germination rate and speed in mung bean seeds. This means that a higher percentage of seeds may germinate, and they may do so more quickly compared to non-primed seeds.

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