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Nanopriming as an Effective Tool for Reducing Heavy Metal Toxicity in Rice

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Abstract

Heavy metals such as Arsenic and cadmium, present in the environment in different oxidative states, affect the productivity and quality of the rice seeds. Seed priming is an effective way to deal with this problem. Seed priming uses treatments to improve seed germination and thus potentially increase growth and yield. It is a low-cost, environmentally friendly, effective seed treatment method. The type of seed priming done in this study was seed nano-priming. 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 and is considerably more effective method compared to all other seed priming methods. Ferrous Nanoparticles have been used in this study for seed nano-priming. Seeds were primed with two concentrations of Fe NPs – 5mg/ml and 10mg/ml. Apart from this, seeds were also primed with deionised water and phytochemical to check the effectiveness of nanoparticles compared to other types of priming. The study shows promising results for the growth of Nano primed rice seeds in the presence of arsenic / cadmium stresses in the environment.

Key Words: - Nanoparticles, arsenic stress, cadmium stress, phytochemicals, seed priming, hydro priming, surface sterilisation , Seed nano priming , root length, shoot length, fresh weight, dry weight, abiotic stress , seed germination

Introduction

Rice (Oryza sativa L.) belongs to the grass family (Poaceae). The domestication of rice by mankind is one of the most significant events in the history of human agricultural advancement (Khush 1997; Molina et al. 2011; Huang et al. 2012). It is one of the world's vital staple grains and plays a crucial role in the entry of mineral nutrients into the food chain (Ali et al. 2018a). Rice produced in rain-fed conditions are often susceptible to a various of biotic and abiotic stresses where mainly the abiotic stresses are the primary factor affecting its growth and yield (Gao et al. 2007). The abiotic stresses include extreme temperature, drought, submergence, salinity, iron toxicity, nutritional deficiencies, and heavy metal contamination (Wu et al. 2014; Li and Ali 2017). The presence of naturally occurring unwanted metalloids such as Arsenic(As) and metals such as Cadmium in flooded paddy soil poses a significant threat to rice production and consumers who depend on rice as their primary staple food (Murugaiyan et al. 2019). Conventionally, rice is produced in flooded paddy fields, which can translocate unwanted class I carcinogenic arsenic metalloid into the straw and grains (Sayan et al. 2012). An accumulation of arsenic in the rice plant negatively affects plant performance and also threatens the health of consumers and livestock (Carbonell-Barrachina et al. 2015). Cadmium toxicity decreases seed germination, growth, mineral nutrients, photosynthesis, and grain yield. It also causes oxidative stress and genotoxicity in

rice (Rizwan, M., Ali, S., Adrees, M. *et al.* 2016). Since we are likely to see a rise in world population in the coming decades, keeping rice production sustainable remains a crucial challenge (Ali et al. 2018a). To reduce the uptake of arsenic and cadmium in rice plants, we have used nanoparticle mediated seed priming.

Seed priming treatment is done before sowing seeds, which involves hydration of seeds plentiful enough to enable metabolic events before germination to take place, although preventing radicle emergence to occur (Pawar and Laware, 2018). Seed priming improves seed performance, ensures uniformity and better establishment, enhances the yield in diverse environments, greater tolerance to environmental stress and helps to overcome dormancy (Raj, A.B. and Raj, S.K., 2019). This is a low cost, low risk technology. In this study, seeds are primed using nanoparticles (particles having any one dimension less than 100 nm).

Nano priming is a new method of seed priming with nanoparticles (NPs) such as zinc oxide, iron oxide, titanium dioxide, silver nanoparticles etc. Fertilizers or nutrients applied to plants are not utilized by them as they get drained away or are broken down by exposure to light and water. However, Nanoparticulate material/nutrient delivery to plants provide adequate and restricted use of nutrients/ macromolecules at a specific site required for enhancing plant growth. (Pawar and Laware, 2018). We have used Ferrous nanoparticles in this study for SNP.

One of the main reason for using ferrous nanoparticle over gold or silver nanoparticle is its economic viability. Also Iron oxide proves to be a source of iron for plant development and synthesizes siderophores. Iron stimulates plant growth by activating several enzymes, RNA synthesis and improving the performance of photosystems in plants (Pawar and Laware, 2018).

Phytochemicals are naturally occurring compounds and have been used for centuries to treat and prevent many illnesses. Several phytochemical compounds have been shown to have potent bioactive moieties with a few even showing promise as anticancer molecules through the modulation of several biochemical pathways and processes associated with oncogenesis (Freidus et al. 2020). Priming using phytochemicals was also done for comparison with nano priming.

The main objective behind carrying out this study was to observe how ferrous nanoparticles primed rice seeds responded to arsenic and cadmium stress individually. The experiment also contains seeds which are hydro primed and phytochemical primed to evaluate the potentiality of different seed priming approaches for increasing seed germination and seedling vigor to combat different abiotic stresses under field condition (here arsenic and cadmium stress).

3. MATERIALS AND METHODS

3.1 Preparation of Ferrous Oxide (FeO-NPs) Nanoparticles

3.1.1. Materials required: -

1. Double Neck round bottom flask
2. Magnetic stirrer and heater
3. Stand
4. Borosil 12inch bowl
5. Upper part of Soxhlet apparatus for maintain the reverse vapor pressure.
6. Thermometer
7. Silicon oil
8. Micro-pipette
9. 50ml Falcon
10. Magnetic bead

Chemicals used: -

1. FeCl3 (Ferric chloride)- 50ml, 0.05M
2. NaOH (Sodium hydroxide)- 50ml, 0.5M
3. Milli-Q Water

3.1.2PROTOCOL:

- Prepare 50ml of 0.05M Fe salt (FeCl3) and 50ml of 0.5M NaOH(Fig2).
- In a double neck round bottle flask pour the FeCl3 and place
- on the Magnetic stirrer and Heater. Then fix the experimental setup as described in Fig. 1
- Place the magnetic bead in the 0.05M FeCl3 solution and maintain the temperature between 70°C - 80°C.
- While heating add the 0.5M NaOH dropwise using the micropipette.
- Continue stirring for at least 4-6 hours.
- After 4-6 hours, put the heater off and keep it overnight at Room Temperature.

Experimental setup

- Then the very next day, centrifuge the solution at 10,000 rpm for 10mins (3-4 times, if necessary increase the time and speed accordingly) in a 50mL falcon. Discard soup and wash the pellet with Milli-Q water and vortex it necessarily.
- Repeat the previous step (2-3 times).
- Discard Supernatant and dry the pellet inside a Hot Air Oven at 80°C for 5-6 hours.
- After proper drying scrape the nanoparticles with spatula from the falcons and make the powder of NPs by using a clean Morter pestle.
- Store the powdered NPs in a micro-centrifuge tube for future use.

3.2 Surface Sterilization of Rice seeds

The variety of rice used in this study are IR64 which is a high-yielding and high-quality indica variety that is widely adapted to tropical lowland growing conditions. The rice seeds, husked or de-husked, are taken in falcon tubes. A small amount of liquid detergent is added to the tubes along with water and shaken for few minutes. The seeds are then cleaned with running water until no lather is present. After discarding the tap water, the falcon tubes were taken under laminar hood and Sodium Hypochlorite was added to the seeds. These seeds are then washed with it by gentle inversion for about 10 minutes. Repeat the steps if necessary. Sodium Hypochlorite also known as bleach ensures complete sterilization of these rice seeds. The seeds are kept for drying inside the laminar hood after a final wash with autoclaved water to remove traces of Sodium Hypochlorite.

3.3 Process of Phytochemical Extraction

Phytochemicals are extracted from the shoot of the plant. The desired plant is taken and all its unnecessary parts are removed. The shoot is then dried and grinded into a powder using mortar and pestle. The solution was made by dissolving 2.5g of this powder in 35 ml of distilled water. The solution was kept on magnetic stirrer at 66o rpm at 24℃ for 4-5 hours. After this, is was filtered twice using filter paper and centrifuged twice at 1000 rpm for ten minutes. This solution was stored in the freezer. Later it is filtered using membrane syringe filter.

3.4 Priming

Priming was done in four setups: -

1) Rice seeds were primed with deionised water.
2) Rice seeds were primed with phytochemical previously extracted.
3) Rice seeds were Primed with Fe NPs of concentration 5mg/ml.
4) Rice seeds were Primed with Fe NPs of concentration 10mg/ml.

Previously Sterilized rice seeds were added to four different conical flasks. Flask 1 had deionized water. Flask 2 had phytochemical. Flask 3 and 4 ha Fe Np solution of concentration 5 mg/ml and 10 mg/ml respectively. These setups were cotton plugged and kept in shaker incubator for 24 hours at 28℃ and 60 rpm. After priming, the seeds were air dried to their original moisture content.

3.5 Plate Preparation

Ten water-agar plates were prepared. Five plates where Arsenic stress was added. The rest five plates had cadmium stress. To prepare water-agar plates, 12 g of Agar powder was dissolved in 1000ml of distilled water (1.2%) and poured into ten petri-plates inside the laminar hood. After solidifying the water agar plates were ready. Five plates were prepared having Arsenic of concentration 10mg/L. The rest five plates were prepared having cadmium of concentration 100µM.

3.6 Seed Germination

The primed seeds are then taken and placed in the water-agar plates prepared in such a way that the embryo is in contact with the media. Unprimed seeds were used as controls. Around fifteen seeds were placed in each plate using forceps carefully. After placing, the plates are covered with micropore tape and cultivated in a dark growth chamber at 28℃ for 7 days.

The setups were: -

1) 2X Control (Water agar plates with unprimed seeds; one with arsenic stress and another with cadmium stress)
2) 2X Hydro (Water agar plates with hydro-primed seeds; one with arsenic stress and another with cadmium stress)
3) 2X Phyto (Water agar plates with seeds which were primed with phytochemical; one with arsenic stress and another with cadmium stress)
4) 2X Fe NP -5mg/ml (Water agar plates with seeds primed with Fe NPs (5mg/ml conc.); one with arsenic stress and another with cadmium stress)
5) 2X Fe NP – 10mg/ml (Water agar plates with seeds primed with Fe NPs (10mg/ml conc.); one with arsenic stress and another with cadmium stress)

4. Results

4.1 Germination Percentage

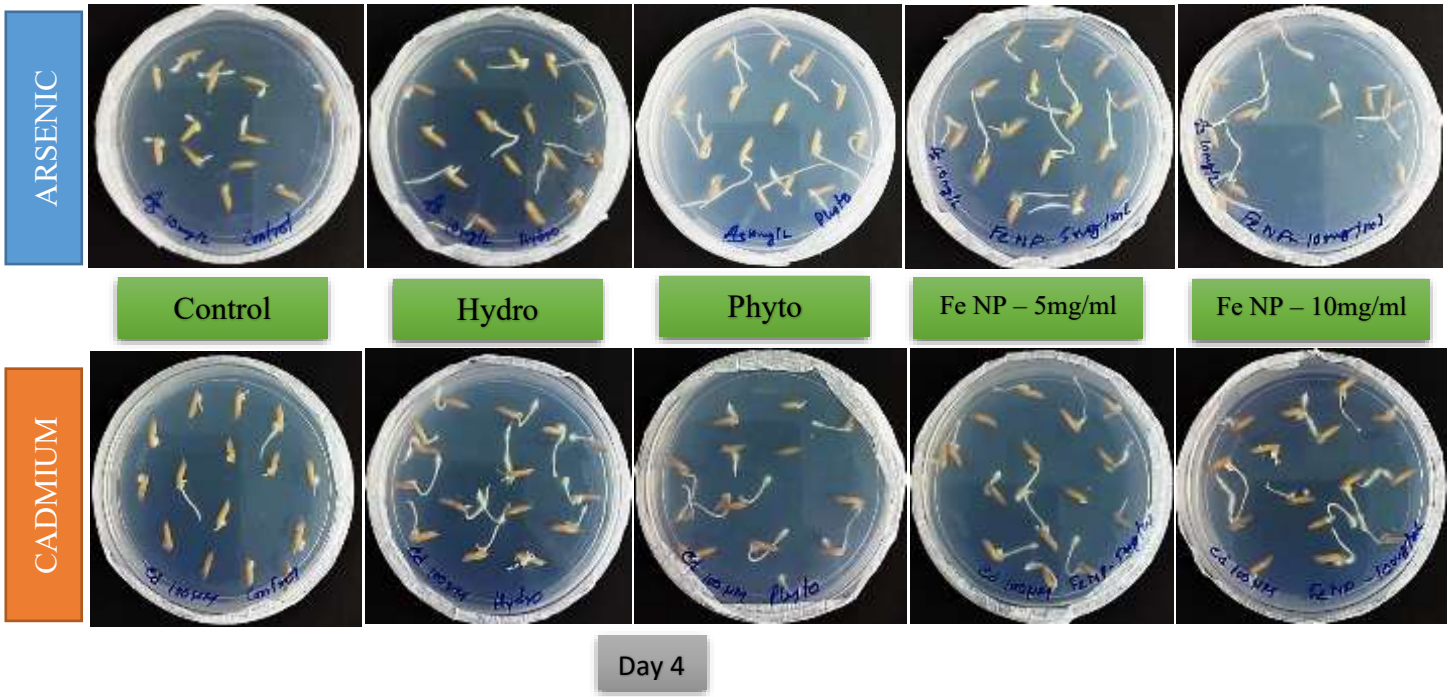
The numbers of germinated seeds were recorded every day. Germination percentage was calculated every day. The germination percentage gave us an idea about the time course of seed germination. It also gave us an estimate of the potential field performance of the seeds.

$$\text{Germination Percentage(GP)} = \frac{Total\ number\ of\ germinated\ seeds}{Total\ number\ of\ seeds}\ X\ 10$$

The germination percentage for individual plates were calculated.

Day 4 (Table 1)

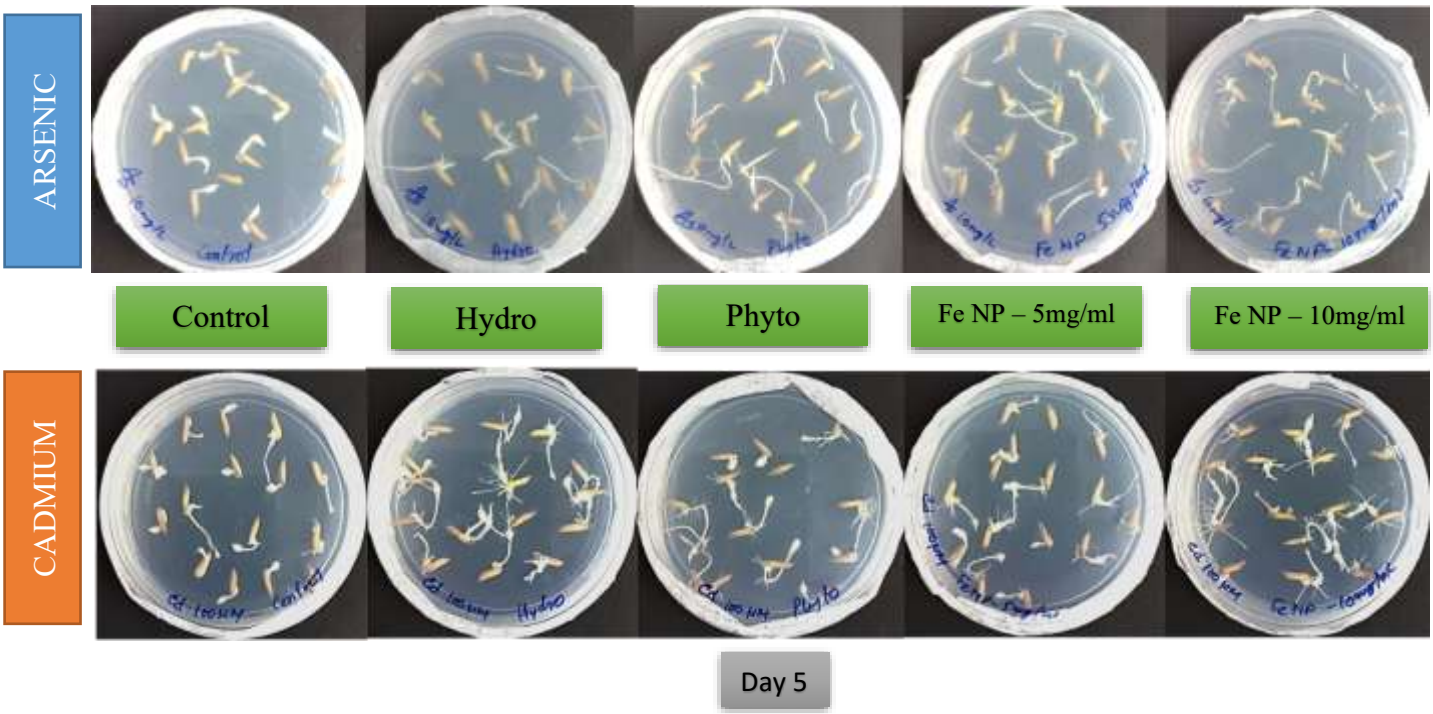
Set ups	GP of Seeds Under Arsenic Stress(%)	GP of Seeds Under Cadmium Stress (%)
Control	80	100
Hydro	86.66	100
Phyto	93.33	93.33
Fe NP - 5mg/ml	93.33	86.66
Fe NP- 10mg/ml	100	100



Day 4

Day 5(Table 2)

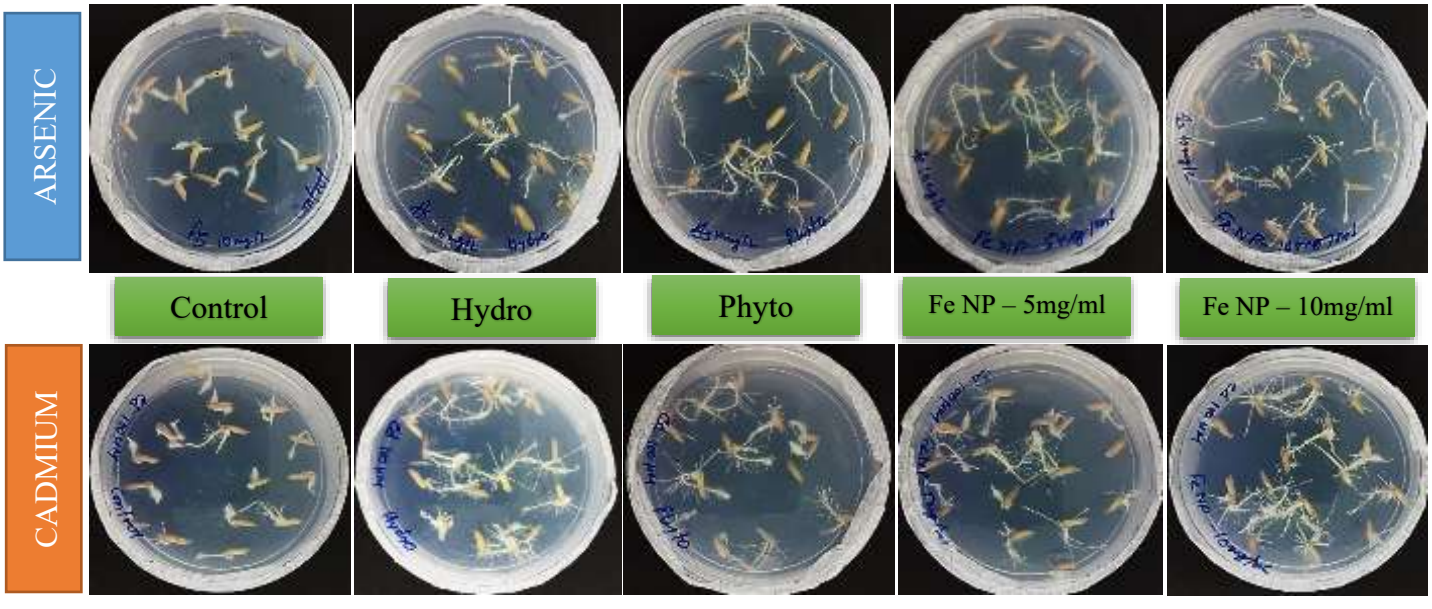
Set ups	GP of Seeds Under Arsenic Stress(%)	GP of Seeds Under Cadmium Stress (%)
Control	86.66	100
Hydro	86.66	100
Phyto	93.33	93.33
Fe NP - 5mg/ml	100	93.33
Fe NP- 10mg/ml	100	100



Day 5

Day 6(Table 3)

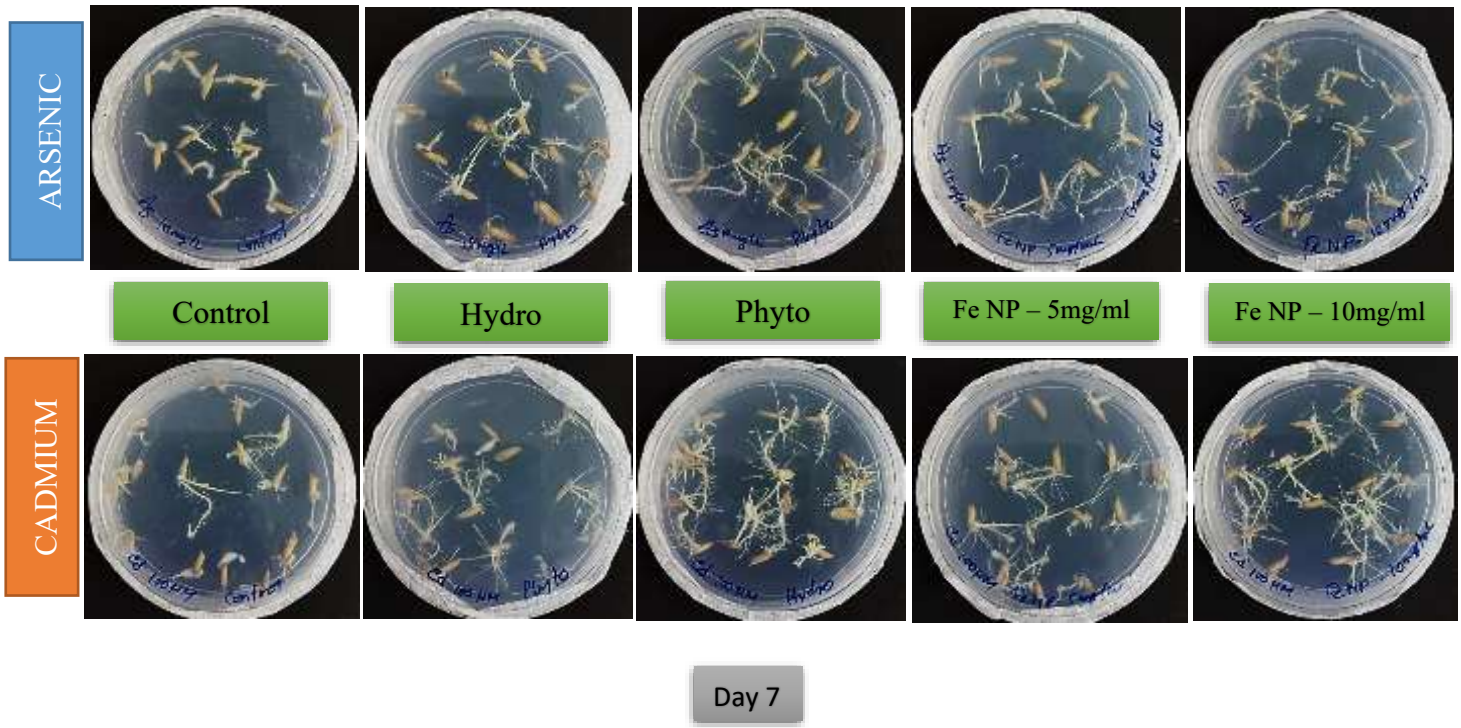
Set ups	GP of Seeds Under Arsenic Stress(%)	GP of Seeds Under Cadmium Stress (%)
Control	100	100
Hydro	86.66	100
Phyto	93.33	93.33
Fe NP - 5mg/ml	100	93.33
Fe NP- 10mg/ml	100	100



Day 6

Day 7(Table 4)

Set ups	GP of Seeds Under Arsenic Stress(%)	GP of Seeds Under Cadmium Stress (%)
Control	100	100
Hydro	86.66	100
Phyto	93.33	93.33
Fe NP - 5mg/ml	100	93.33
Fe NP- 10mg/ml	100	100



Day 7

4.2 Root and Shoot Length

After 7 days of germination, five seedlings were randomly selected from each replicate for measurement of shoot length and root length. The mean was calculated. Shoot length and Root length were measured with a ruler. The best three readings were taken and mean was found out.

Table 5

Set ups	Shoot length(in mm)			Mean
	1st sample	2nd sample	3rd sample	
As- Control	15	20	11	15.3
As- Hydro	31	24	21	25.3
As- Phyto	30	29	27	28.6
As- Fe 5mg/ml	27	24	23	24.6

As- Fe 10mg/ml	28	23	19	23.3

Table 6

Set ups	Root length(in mm)			Mean
	1st sample	2nd sa	3rd sample	
As-Control	14	14	12	13.3
As- Hydro	24	22	14	20
As- Phyto	35	33	32	33.3
As- Fe 5mg/ml	37	35	28	33.3
As- Fe 10mg/ml	29	26	20	25

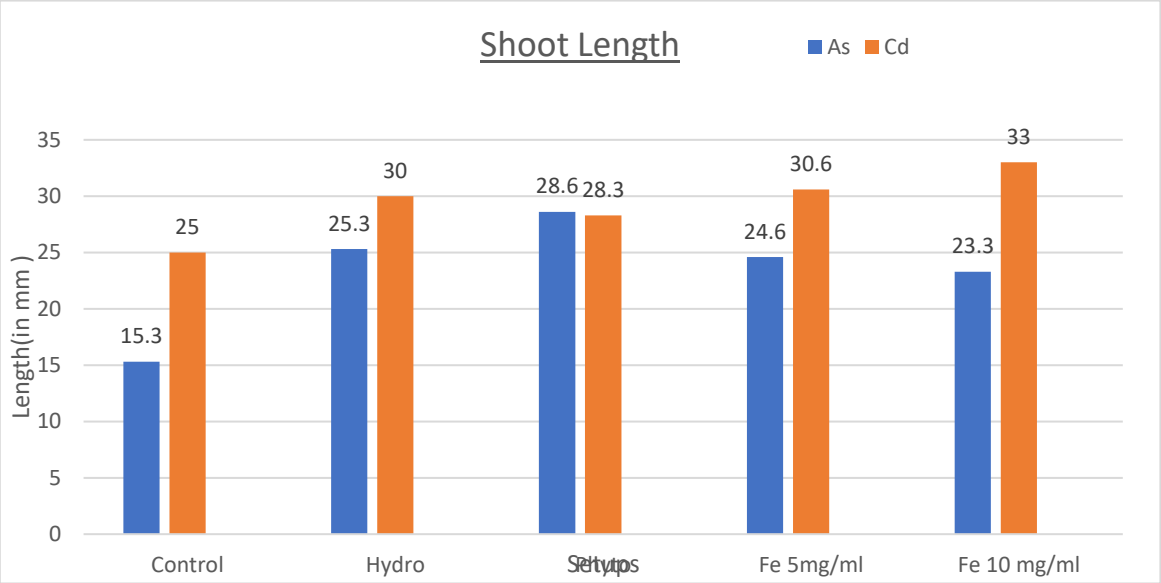
Table 7

Set ups	Shoot length(in mm)			Mean
	1st sample	2nd sample	3rd sample	
Cd- Control	30	28	17	25
Cd- Hydro	34	31	25	30
Cd- Phyto	30	28	27	28.3
Cd- Fe 5 mg/ml	36	31	25	30.6
Cd- Fe 10mg/ml	36	35	28	33

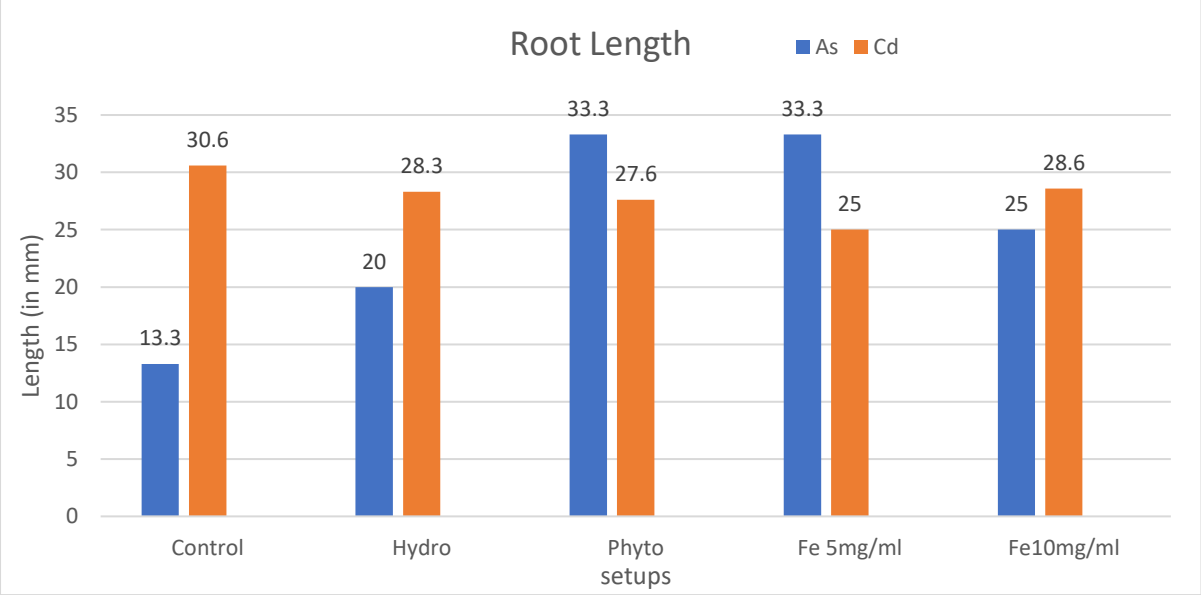
Table 8

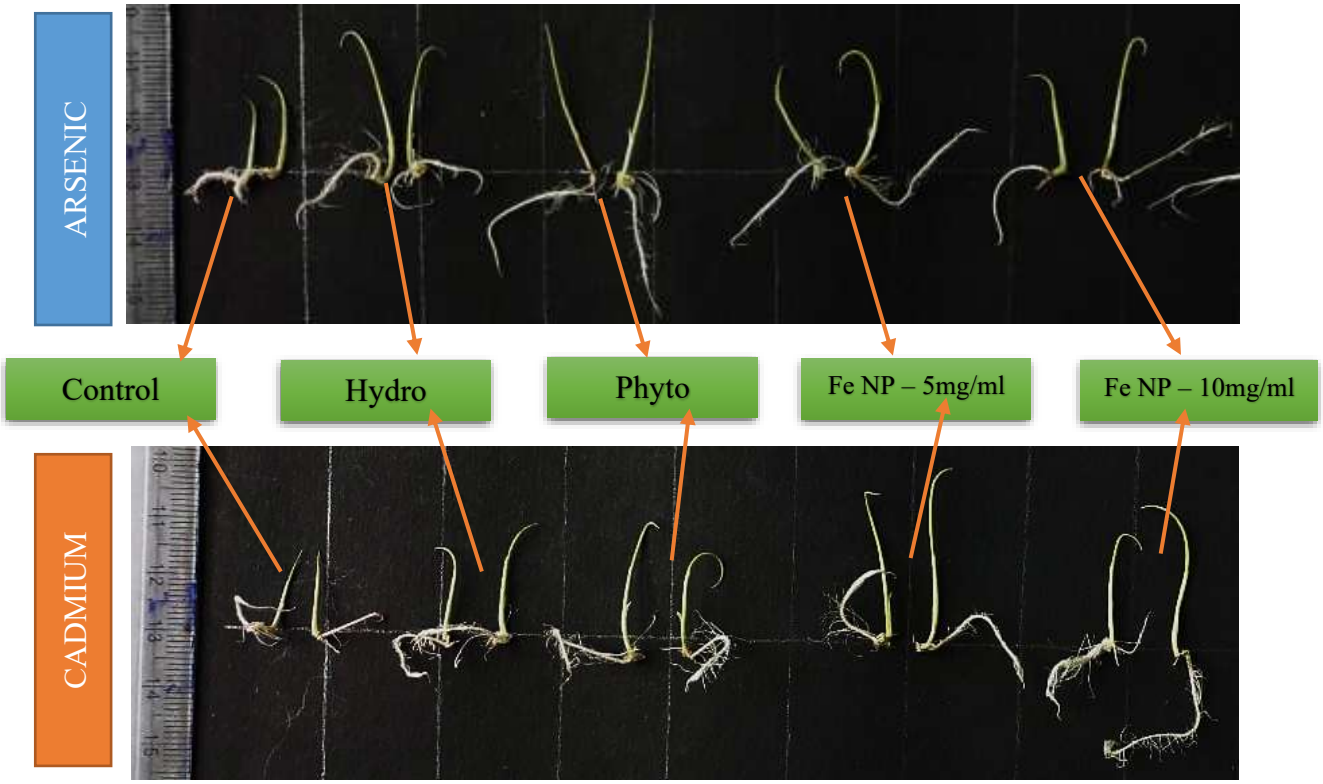
Set ups	Root length(in mm)			Mean
	1st sample	2nd sample	3rd sample	
Cd- Control	37	33	22	30.6
Cd- Hydro	30	28	27	28.3
Cd- Phyto	30	27	26	27.6
Cd- Fe 5mg/ml	27	25	23	25
Cd- Fe10mg/ml	33	25	16	24.6

Comparison of shoot length between seeds in arsenic stress and seeds in cadmium



Comparison of root length between seeds in arsenic stress and seeds in cadmium



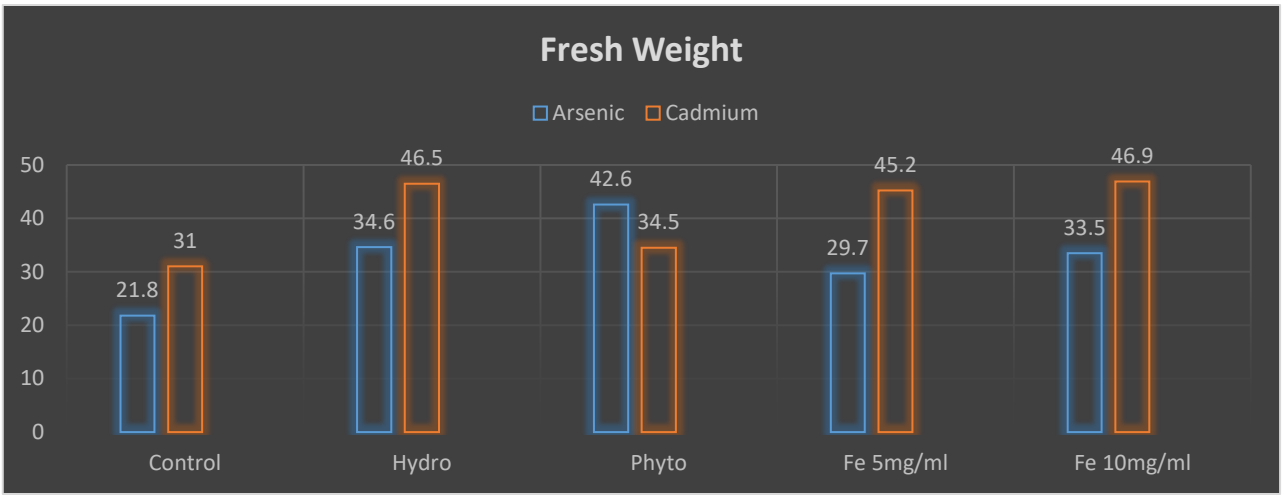


4.3 Dry weight and Wet weight

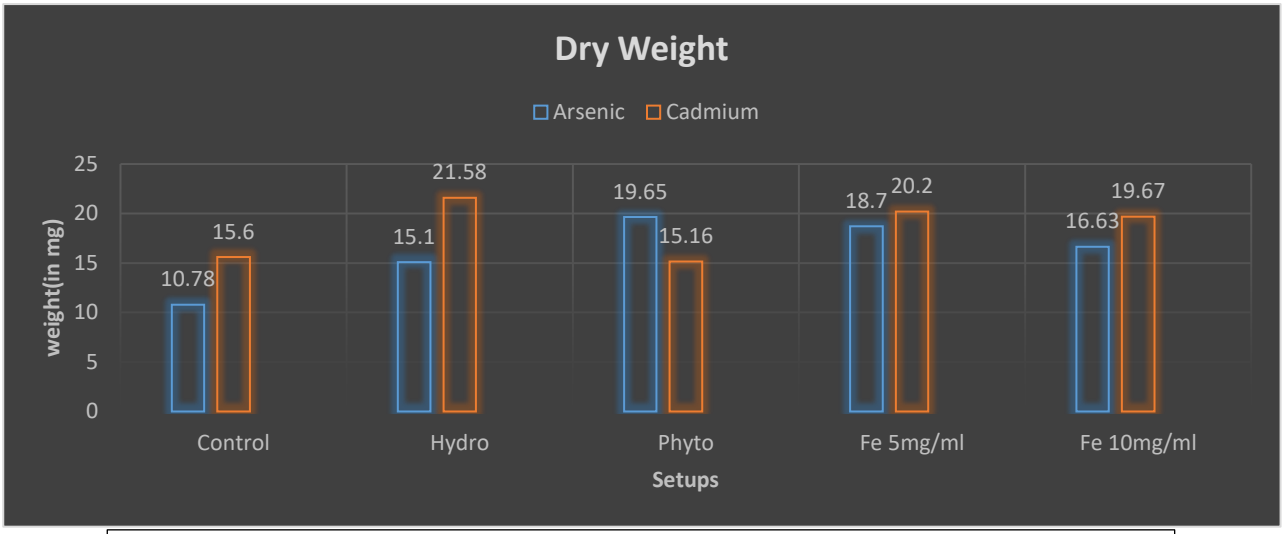
The Fresh weight (wet weight) and dry weight was calculated. Shoot and root tissues were dried to a constant weight at 80°C in an oven. Fresh and dry weights were measured using analytical balances. The Fresh weight when compared to the dry weight gives an idea about the water imbibing capacity of the seeds.

Set up	Seeds Under Arsenic Stress		Seeds Under Cadmium Stress	
	Wet Weight(mg)	Dry Weight(mg)	Wet Weight(mg)	Dry Weight(mg)
Control	21.8	10.78	31	15.6
Hydro	34.6	15.1	46.5	21.58
Phyto	42.6	19.65	34.5	15.16
Fe NP - 5mg/ml	29.7	18.7	45.2	20.2
Fe NP- 10mg/ml	35.5	16.63	46.9	19.67

Table 9



Difference in Fresh weight of seeds under Arsenic and cadmium stress respectively



Difference in Dry weight of seeds under Arsenic and cadmium stress respectively

5.Discussion

5.1 Analysis of Germination Percentage

When the germination percentage was calculated in Day 4, only the seeds primed with Fe NPs of conc. 10 mg/ml had germination percentage of 100 in both arsenic and cadmium stress. Followed by it, seeds primed with phytochemical showed germination percentage of 93.33 in both arsenic and cadmium stress. Fe NP 5 mg/ml had germination percentage of 93.33 in arsenic stress and 86.66 in cadmium stress. Hydro primed seeds and control had around similar germination percentage which is around 80% in arsenic stress and 100% in cadmium stress. Seeds primed with Fe NP – 10mg/ ml did exceptionally well where all of the seeds germinated in arsenic and cadmium stress.

On comparing the germination percentage of day 4 to day 7, only slight changes are observed. Germination percentage of all the setups remain same except for Fe NP- 5mg/ml set up and control. The germination percentage of seed primed with Fe NP -5 mg/ml increased from 93.33 to 100 in arsenic stress and from 86.66 to 93.33 in cadmium stress. Where as in case of control the GP increased from 80 to 100 in arsenic stress.

5.2 Analysis of Shoot and root length

In Arsenic stress, the shoot length of seeds primed with Fe NP of conc. 5mg/ml and 10mg/ ml were 60% and 52% greater than that of control respectively. Hydro primed seeds also showed a shoot length which was 65% greater than control. The best result was seen in phytochemical primed seeds. The shoot length was 87% greater than that of control. In Cadmium stress, the shoot length was not much greater when compared to the control. The Fe NP primed seeds of conc. 5mg/ml showed a 22% greater shoot length than that of control whereas Fe NP primed seeds 0f conc. 10 mg/ml showed shoot length which was 32 % greater than that of control. The phytochemical primed seeds showed shoot length which was 13% greater than control. Hydro primed seeds had 20% greater shoot length when compared to the control setup. The best result was seen in Phytochemical primed seeds and Fe NP of conc. 5mg/ml in arsenic stress. In cadmium seeds primed with Fe NP of conc. 10mg/ml showed the best result.

When the root length was compared with respect to control, Seeds which were under Arsenic stress showed excellent result. But it was opposite in case of seeds under cadmium stress. The root length of control in cadmium was the greatest when compared to other primed seeds which should not have been the ideal case. In Arsenic stress, Phytochemical primed seeds and seeds primed with Fe NPs having conc. 5 mg / ml had root length 1.5 folds greater than that of control. Seeds primed with Fe NPs having conc. 10mg/ml showed 87% greater root length when compared to control setup. Hydro primed seeds also showed 50% greater root length when compared to control setup.

In conclusion we can say that seeds that were primed with Phytochemical and the seeds that were primed with Fe NPs having conc. 5mg/ml showed the best result when subjected to arsenic stress. While in cadmium stress, seeds primed with Fe NPs having conc. 10mg/ml showed better results compared to the other setups.

5.3 Analysis of Fresh and dry weight

When the Fresh weight and dry weight was compared to get an idea of the water imbibing capacity of the seeds, it was seen that the maximum water holding capacity under arsenic stress was in Phytochemical primed seeds. Followed by it was the Hydro primed seeds. Seeds primed with Fe NPs of conc. 10mg/ml had better moisture retention capacity that seeds primed with Fe NPs of conc. 5 mg/ml under arsenic stress. When compared with control the water holding capacity of all other primed seeds, except seeds primed with Fe NPs having conc. 5 mg/ml, were much better in arsenic stress.

Under Cadmium stress, the maximum water holding capacity was seen in seeds primed with Fe NPs having conc. 10mg/ml. All other primed seeds also showed near about similar water retention capacity. When compared with control, the water holding capacity of all other primed seeds were much better in cadmium stress.

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