Invigoration and Root Growth Enhancement of Mung Bean Seeds Through Pre-Treatment With *Allium Cepa* 30C – An Agrohomeo Study

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Abstract:-**Keeping the seed health well during storage is a very serious problem as this makes old seeds incapable to fully germinate. We report here for the first time that pre-treatment of two year-old mung bean seeds with a low cost homeopathic medicine Allium cepa 30C is effective under normal laboratory conditions in invigorating the seeds as realised by their higher growth rate, as well as enhancement of the root growth from these seeds. We also showed that the medicine does not have any side effect, thus making this study very useful for farmers in the horticultural as well as agricultural sectors.**

Key words: Invigoration, root growth, pre-treatment, viability, Seed electrolyte, *Allium cepa*

I. INTRODUCTION

Maintenance of seed viability during storage is a serious problem the horticultural as well as agricultural sectors face in the tropical countries. During storage, high temperature and relative humidity accelerates the deterioration of seed membrane through loss of water from membrane tissues which in turn damage the cells [1]. Ascorbic acid regulates the hydrogen peroxide [H2O2] level in water stressed tissues. H2O2 is a toxic metabolite which is accumulated in water stressed tissues and increase membrane permeability [2]. Over the years various methods have been adopted by scientists like hydration-dehydration techniques as well as treatment of seeds with chemicals, vitamins, fungicides, plant growth regulators, antioxidants etc [3-7]. However, farmers prefer to choose the techniques which are easy, cost effective, non toxic or non hazardous and do not take much time.

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We realize that homeopathic medicines are universally used for centuries, because of their negligible side effects, low cost, easy availability and easier applicability [8-13]. The effects of homeopathic medicines have been studied in details mostly in animal systems [14-17]. However, plants being the silent receptors of the ecosystem, we were interested to find the effect of homeopathic medicines specially on invigoration of plants. Recently researchers are engaged themselves in the field of agrohomeo studies [18-22].

In the present study we have used for the first time a homeopathic medicine, Allium cepa 30C, a well known homeopathic medicine, commonly used for treatment of cold, to study its effect on two year old mung bean seeds.

Our experiments conducted on petriplate using mung bean seeds, pre-treated with Allium cepa 30C, indicated that the drug improved the seed membrane health and showed that they not only could invigorate the seeds as realised by their higher growth rate, but they also enhanced the root growth from these seeds.

We also showed that the medicine does not have any side effect, thus making this study very useful for farmers.

II. MATERIALS & METHODS:

A. Seed collection:

Certified two-year old mung bean seeds were collected from a local farm of Hooghly District. The germinability of the seeds was 65% on the average.

B. Pre-treatment:

Collected seeds were surface sterilized with 0.1% HgCl2 for 1 min and then treated with Allium cepa 30c (diluted with double distilled water 1:50 before use). As the growth of onion's root tip cells are much high and as Allium *cepa* is prepares from onion so for our purposes we have chosen *Allium cepa* in its diluted form i.e. *Allium cepa* 30c. Equal number of seeds was soaked in 100 ml of *Allium cepa* 30c solution and same amount of distilled water (DW) for comparison. In both the cases treatments were continued for 6 hrs. After that the seeds were separately surface dried with blotting paper and dried back to their original weight under sun. The control set was not pre-treated either with DW or *Allium cepa* 30c. Finally the three sets of seeds were stored in perforated paper bags and experiment started after 10 days. The treated seeds were designated as follows

S_0 – Control

S_1 – DW pre-treated

S_2 – *Allium cepa* 30C pre-treated

C. Germination technique:

Before starting the experiment 100 individual surface sterilized seeds were transferred to petridishes containing filter paper moistened with 15 ml of DW or diluted *Allium cepa* 30C as the case may be. Following the rule of International Seed Testing Association (ISTA) germination data were recorded after 24 h interval [23]

D. Measurement of different parameters:

Germination percentage of three sets were recorded carefully. Time taken for 50% of germination i.e. T_{50} was recorded after repeating germination process thrice with three replication in each case. [Table I].

Dry weight accumulation was recorded after 15 days growth of seedlings. The shoot and root growth of the seedlings were also recorded separately.

Treatment	T50 of germination (h)	Percentage of Germination	Dry weight accumulation (mg g^{-1} fresh wt)
S_0	24 ±2.5	68% ±3.0	165±4.2
S_1	22 ±2.0	75%±3.5	176±3.6
S_2	18±3.1	94%±3.0	194±4.0

Table I: T_{50} of different treatments and the dry wt accumulate in 15 days' old seedlings.

Phytotoxicity of *Allium cepa* 30c was evaluated by calculating relative root elongation (E) and germination index (GI), which actually is used for agricultural purposes as an indicator of phytotoxicity in soil [24]. The percentage of E and GI were calculated according to the standard method of Batish et al [25].

Treatment	Length of 15 days old seedling (cm)		E(% ± 20)	GI (% ±30)
	Shoot length (±2.0)	Root length (±2.6)		
S_0	15.2	7.0	78.55	80.50
S_1	15.4	7.2	85.60	86.80
S_2	15.5	8.8	101.50	99.50

Table II: Length of seedlings and GI and E% of different treatments.

For measurement of electrolyte leachate (EL), 25 seeds of each replicate were taken in 100 ml beaker containing 25 ml of DW at 27 ± 1ºC. After 24 h of treatment the seeds were removed and the electrolytes were taken for different experiments. Electrical conductivity was determined by conductivity meter. Soluble carbohydrate and soluble amino acid were determined by the procedure of McCready et al and Moore and Stein, 1948 respectively [26, 27]. [Table III].

Dehydrogenase activity was analyzed by using 20 de-husked mung bean seeds imbibed in DW for 24 h in sterilized water. The hydrogen atoms released by the dehydrogenase enzyme which are involved in respiration, reduce tetrazolium to red colour formazan. [Table III].

Treatment	Conductivity	Soluble carbohydrate (µg/g/25 ml)	Free Amino acid (µg/g/25 ml)	Dehydrogenase study in terms of OD (nm ± 2.44)
S_0	210 ±12.50	36 ±3.50	62.12 ±5.50	0.35
S_1	188 ±12.00	29 ±2.65	58.00 ±4.50	0.42
S_2	157 ±7.85	20.50 ±2.20	45.20 ±5.40	0.65

TableIII: Conductivity reading of different treatments and the amount of soluble carbohydrates, free amino acids and the results of dehydrogenase study.

Statistical significance were analyzed by one-way ANOVA with an appropriate multiple comparison test at $p \leq 0.05$.

III. Results and Discussion

T_{50} of germination was 18±3.1 in case of S_2 which indicated the improvement of seed quality through the pre-treatment of *Allium cepa* 30C compared to S_0 which showed T_{50} of 24±2.5 h. After the pre-treatment seed germination percentage increased from 68% (in case of S_0) to 94% (in case of S_2). This was further supported by the enhancement of dry wt. accumulation in case of S_2 in comparison to S_0 and S_1. Earlier researchers have shown that post imbibitions drying actually advances the onset of germination after subsequent rehydration compared with non-dried controls [28]. As the T_{50} of S_2 took less time in comparison to S_0, and S_1, it may be concluded that pre-soaking of seeds in *Allium cepa* 30C and subsequent drying allowed the soaked seeds to be sufficiently advanced in its germination process.

Table II showed the data of E and GI, which indicated that after 15 days, both S_1 and S_2 showed positive effect on E and GI indicating no toxic effect of *Allium cepa* 30C on mung bean plants. Another important thing was that after 15 days of treatment, the overall length of seedlings enhanced in case of S_2 compared to S_0 and S_1. However, soot height remained more all less same but root length enhanced remarkably in case of S_2 (8.8 cm) compared to S_0 (7.0 cm) and S_1 (7.2cm). Thus the treatment is helpful to enhance root growth also.

The proposal that occurrence of membrane lesions might play a significant role in the deterioration of seeds has been supported by the study on solute leaching accompanying a fall in germinability and viability [29]. From Table II it was clear that leached out sugar and amino acids were maximum in case of S_0 which was highly checked in case of S_2 indicating the arrestation of free sugars and amino acids through the seed membrane. This indicates that the potentised homeopathic medicine *Allium cepa* 30C is undoubtedly effective on cell membrane.. On the other hand results of dehydrogenase study indirectly indicated the presence of more living cell in case of S_2 than S_1 and S_o. Actually dehydrogenase study helps to denote the dead and living cell percentage of seed membrane in terms of OD values.

As our overall study showed positive effect on mung bean seed invigoration, the efficiencies of our results were judged through membrane permeability. Conductivity test revealed that seeds leached out maximum in case of S_o

which was highly controlled in case of S_2. Higher leaching of electrolytes is an evidence of seed membrane deterioration. Prevention of leachates indicate that the pre- treatment with *Allium cepa* 30 C checks the loss of potassium, phosphate, sugar, free amino acids, etc through the seed membrane [30]. This effectively improves the membrane permeability which in turns helps to maintain seed health well. Pesis and Timothy, [31] showed that for damaged membrane, conductivity of pooled out electrolytes increased which is proved from our result also as showed in Table III.

CONCLUSION

From our experimental observations, we can conclude that pre-treatment of damaged mung bean seeds through *Allium cepa* 30C can successfully alleviate the storage deterioration, without any significant side effect, through minimizing the seed membrane damage as revealed by their higher growth rate, enhancement of root growth and thus makes this study very promising and useful for farmers in horticultural as well as in agricultural sectors.

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