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Analysis of Biochemical Parameters of *Amaranthus Tristis* During Seed Germination using $Cacl_2$, Bijamrita and Cyanospray

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I. Introduction

A. tristis is recognized as an easy growing, productive, tasty and nutritious leaves (Baquar and Olusi, 1988). The green-colored leaves are grown throughout the world as ornamentals and in Asian countries it is occasionally eaten raw in salads and the soft stems are eaten like asparagus in India. Medicinally *A. tristis* is used externally to treat inflammations, and internally as a diuretic. In Africa *A. tristis* is grown on a limited scale in home gardens or as a commercial vegetable, but it is of little economic significance.

Seed germination and seedling development are well regulated process in plant physiology involving high metabolic activity and generation of reactive oxygen species (ROS). Emergence of the seedling takes place 3–5 days after sowing with fast vegetative development. Like maize and sugar cane, Amaranthus is characterized by the C4-cycle photosynthetic pathway, which means a high photosynthesis at high temperature and radiation and flowering may start 4–8 weeks after sowing. During seed germination, a breakdown of seed reserves, carbohydrates, and protein (Vanderstoep,1981) takes place. Though germination do not increase the protein value of the grain or its biological utilization (Colmenares De Ruiz, 1990), the loss of proteins from the cotyledons could be due to the transport of amino acids to the growing axis or to respiratory loss, or it might result in the accumulation of free amino acids in the cotyledons (Beevers and Spittoesser, 1968) and free amino acid contents are increased during germination period (Ali Al-Heal, 1992).

Bijamrita (Bija-seed; Amrita-elixir) is a traditional biofertilizer especially used for seed treatment. It enhances germination, protects from phytopathogenic infections and increases till ring and plant vigour (Subhash Palekar, 2006). Cyanospray is produced by biodegradation of coir pith using fresh water cyanobacterium (*Oscillatoria annae*) (Malliga *et al.,* 2012).

Amylases are known to play a major role in starch breakdown in cereal seeds (Guglielminetti *et al.,* 1995) and during germination under low oxygen stress, rice seeds are capable of partial degradation of starch into readily fermentable carbohydrates to generate the minimum energy required for growth of the coleoptiles. Starch is a major energy source for developing rice embryos and the seedling can be used to supply energy for growth, and also build up cellulose to make up cell walls for the new cells that are formed as the seed grows.

The key objective of this study is to determine the efficacy of seed germination status of *A. tristis* using calcium chloride, Bijamrita, cyanospray and combination of Bijamrita and cyanospray. The effect of protein, amino acid and amylase during seed germination of *A. tristis* were also analyzed. The main objective, therefore, is an attempt to understand the role of protein, amino acid and amylase during various stages of seed germination in the respective plant. This study certainly helps in understanding the effect of inorganic and organic fertigations on seed germination in *A. tristis* at 12, 24, 36 and 48 hrs.

II. Materials and Methods

a) Calcium Chloride Solution

Different concentrations such as 0.1% to 0.5% of $CaCl_2$ were prepared using distilled water and only distilled water was used for germination study as control.

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b) Bijamrita Solution (Palekar, 2007)

5 Kg of wild cow dung was taken in a cloth and bound by tape and was submerged in 20 L of water for 12 hrs. Simultaneously, 50g of slaked lime was dissolved in 1L of water in separate container and kept stable for overnight. After 12 hrs, this bundle of cow dung was squeezed thrice, thereby: all the essence of cow dung will be drawn to water phase (cow dung extract). 1Kg of soil was dissolved in cow dung extract by stirring it well. To this, 5L of wild cow urine and lime water was added and mixed well. The Bijamrita was applied in different concentrations (12.5%, 25%, 50% and 100%) and used for germination study.

c) Organism and culture condition

A fresh water cyanobacterium belonging to *O. annae* was obtained from the germ plasm of NFMC, Bharathidasan University, Tiruchirapalli, Tamilnadu, India. The culture was maintained in BG 11 medium (Rippka, 1979) at 1500 lux light and 25±2°c with 14/10 Light/Dark cycle.

d) Lignocellulosic Waste

Coir pith was collected from coir industries nearby Srirangam, Tiruchirapalli, Tamilnadu, India.

e) Cyanospay

O. annae with coir pith was inoculated in the ratio 1:10 (dry weight). After 25-30 days of incubation, pellets and supernatant of partially degraded coir pith were separated and the dried pellet was prepared in different concentrations by dissolving pellet in distilled water to be used as cyanospray and without germinated seeds were used for analyzing all the parameter noted as untreated seeds (Abraham Christopher *et al.,* 2007).

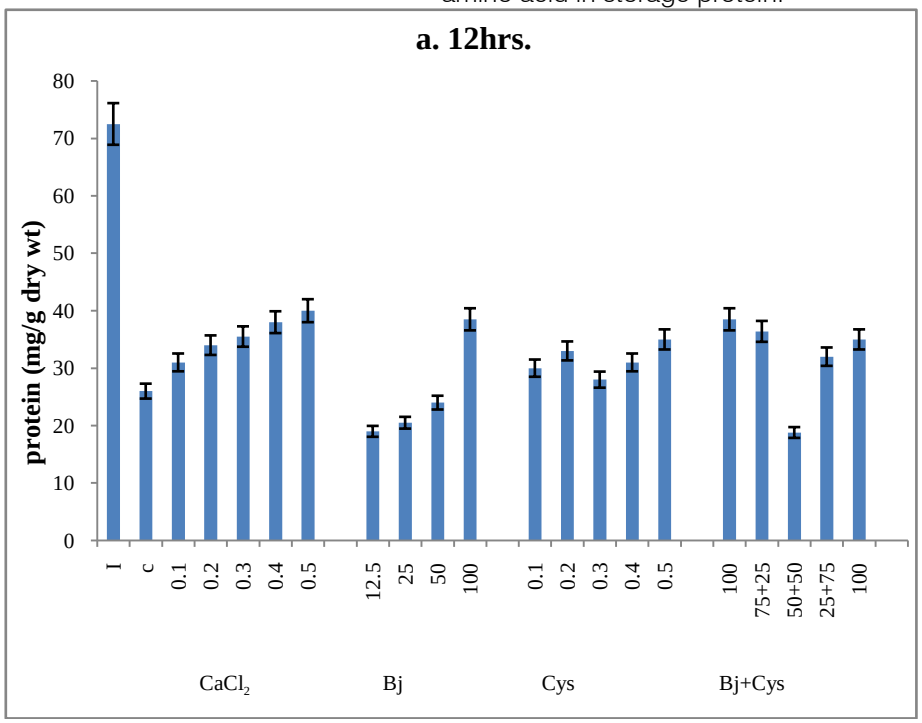
f) Chemical analysis

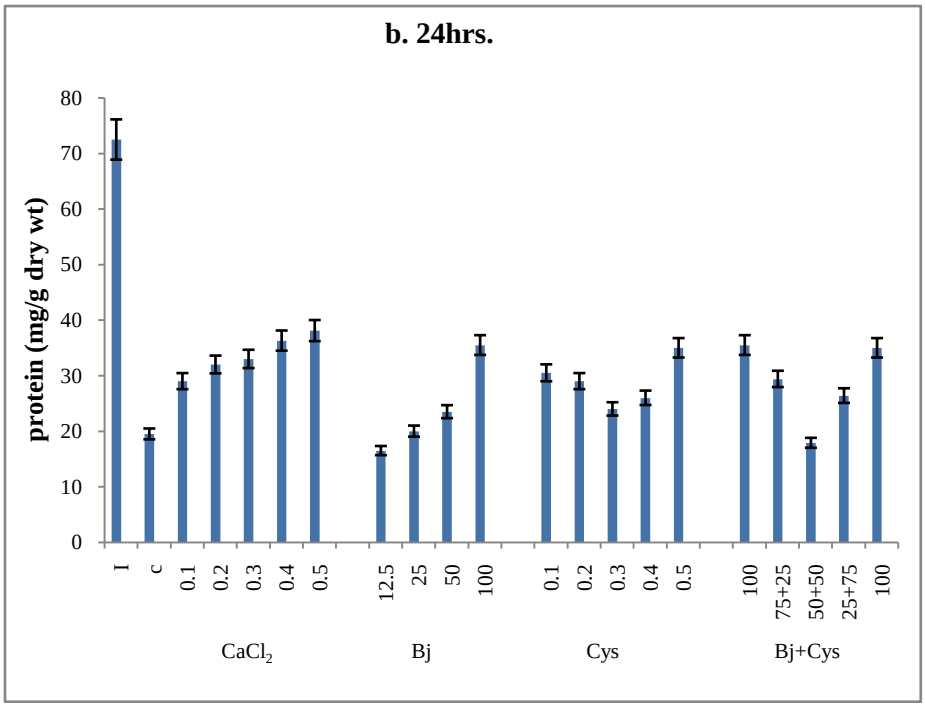
Different biochemical parameters like protein (Lowry *et al.,* 1951), amino acid (Moore and Stein, 1948), and amylase (Katsuni and Fukuhara, 1969) were analyzed at 12, 24, 36 and 46 hrs of germination.

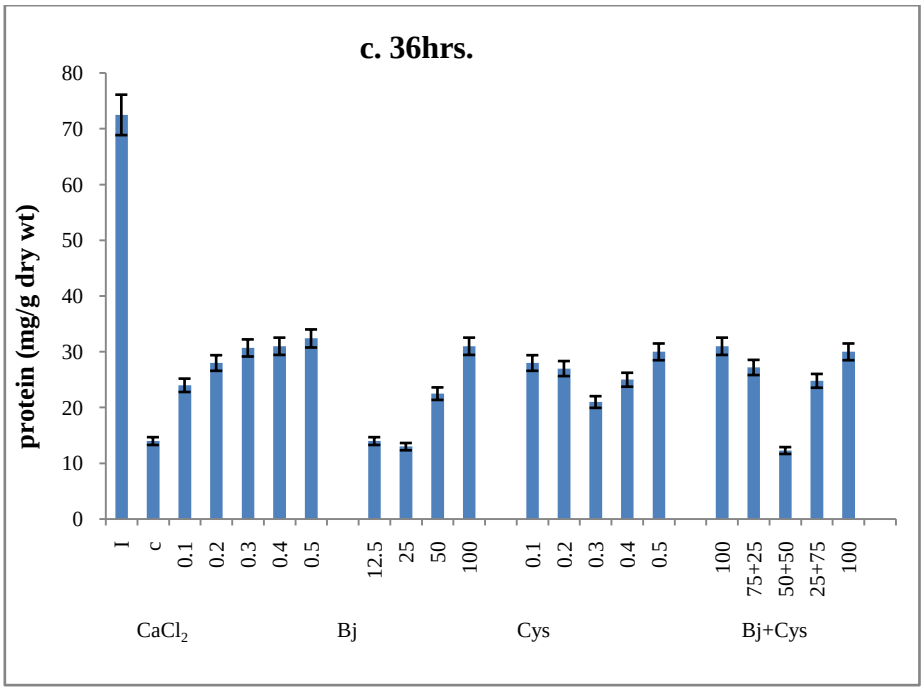
III. Results and Discussions

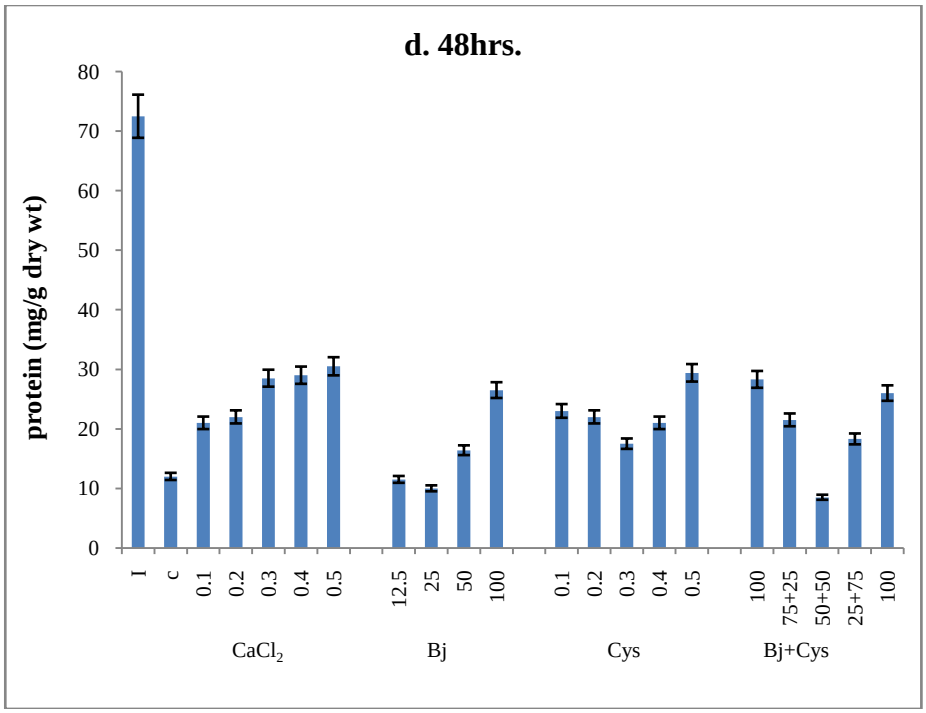
a) Protein

The total protein content was high in untreated seed of *A. tristis* (Fig 1) observed during, germination period but on the contrary, the protein content was decreased at moderate level of 25% Bijamrita and 0.3% cyanospray from 12 - 48 hrs of incubation period. (Fig 1 a-d). Henkel, (1965) reported that calcium chloride lead to redistribution of nutrient reserves resulting in greater internodal length in crop. Sashidhar *et al.,* (1977) reported an increased yield in groundnut when seeds were treated with one per cent calcium chloride for eight hours with high free proline accumulation which is an adaptive mechanism of drought tolerance. However, the maximum reduction of protein content was observed in 48th hr in combination with 25% Bijamrita and 0.3% cyanospray (50:50) preparation when compared with all other treatments (Fig 1.a-d) and the protein content in seeds treated with $CaCl_2$ (0.5%) was found to be higher when compared to all other treatments including control. As the seeds of higher plants accumulate large amounts of storage proteins during seed development and seed maturation, which are mobilized to provide building blocks and energy for seed germination and early seedling growth upon seed germination (Bewley and Black, 1994). During seed development and maturation, storage proteins are broken down into newly synthesized amino acids which are major source of amino acid in storage protein.









-Initial, C-Control, $CaCl_2$-Calcium Chloride, Bj- Bijamrita, Cys- Cyanospray. (Different concentrations in percentage).

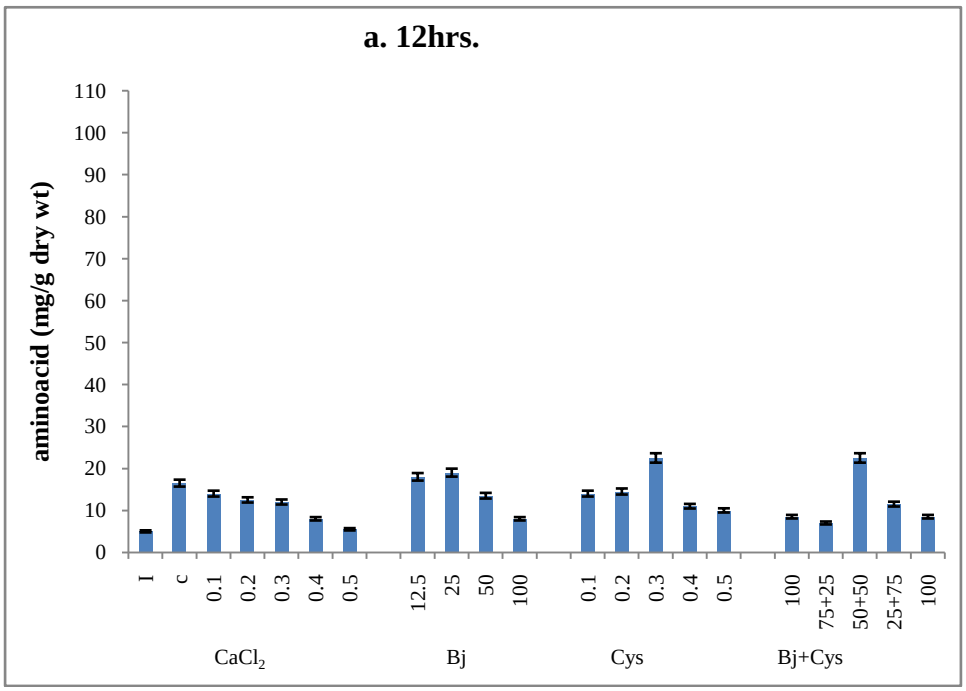
Figure1 : Effect of different concentrations of $CaCl_2$,Bijamrita and Cyanospray on protein content of *Amaranthus tristis* L.

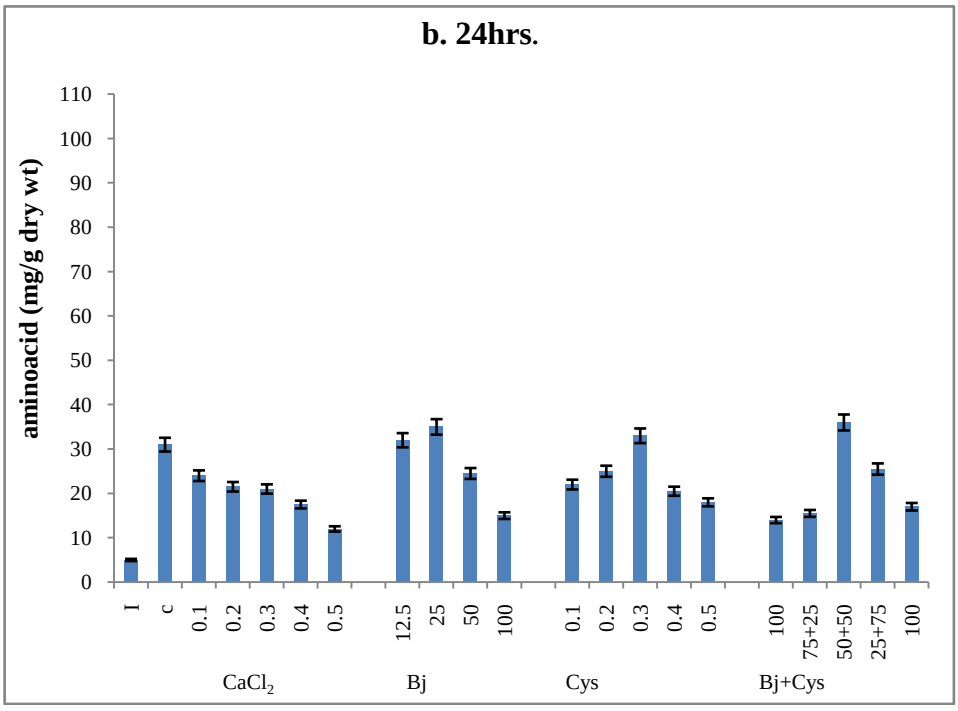
The protein level decreased during germination in *Lupinus leteus L* (Mariusz *et al*., 1992), Australian sweet lupin (Rumiyati *et al*., 2012), *Ceiba pentandra* seeds (Chekuboyina *et al*., 2012), Horse gram seed (Pek Geok Pang *et al*., 2012) and chickpea seeds (Guilherme *et al*., 2005).

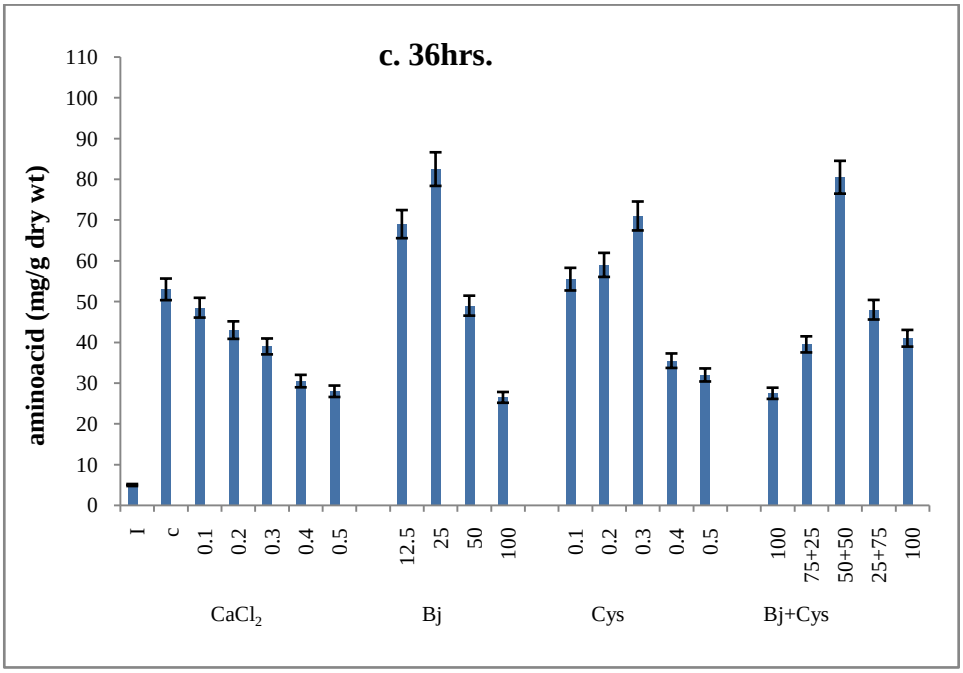
Same results were reported by (Muhammet *et al*., 2012) in the case of safflower (*Carthamus tinctorius* L.) and (Yoshida *et al*., 1997) in *Vigna mungo* cotyledons during germination. Considerable decrease in the protein content was observed in germinating *Lupinus luteus* and *L. angustifolius* (Olczak *et al*., 1992), soybeans (Tan-Wilson *et al*., 1996), Bambara groundnuts (*Voandzeia subterranea* L. Thouans) (Obizaba and Egbuna, 1992), fluted pumpkin (*Telfairia occidentalis* Hook) (Giami *et al*., 1999), and sunflower seeds (*Helianthus annuus*) (Balasaraswathi and Sadasivam, 1997). The loss of proteins from the cotyledons could be due to the transport of amino acids to the growing axis or to respiratory loss, or it might result in the accumulation of free amino acids in the cotyledons. These reports are similar to the results of (Beevers and Spittoesser, 1968) in germinating Peas and in the cotyledons of Mung Bean Seedlings (Kern and Chrispeels, 1978).

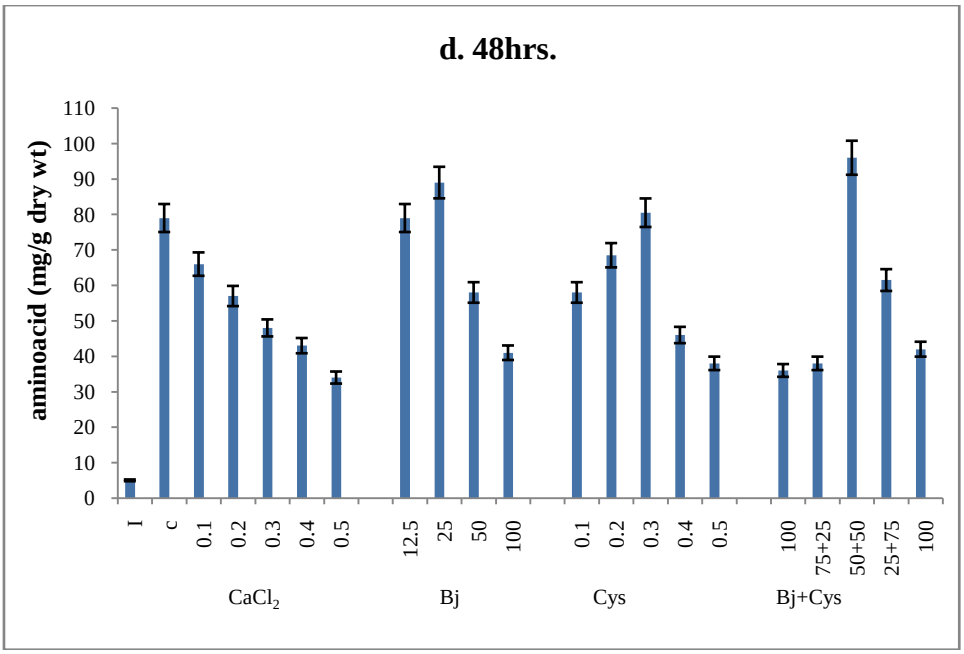
b) Free amino acid

The amino acid content was very low in untreated seeds, but the control and treated seeds during germination period showed the presence of more amino acid content than normal seeds (Fig.2 a-d). From the data *(Fig.2 a-d),* it could be observed that all the treatments during 36-48hrs germination showed increase in amino acid content especially in combined effect of Bijamrita with cyanospray (50:50) *(Fig.2 a-d)* at 48th hr. When compared to all other treatments, increased amino acid content in certain concentrations of organic fertigations at 48th hr can be due to induction of germination, which results in hydrolysis of protein for their growth thereby releasing amino acids for their growth.









-Initial, C-Control, $CaCl_2$- Calcium Chloride, Bj- Bijamrita, Cys- Cyanospray
(Different concentrations in percentage)

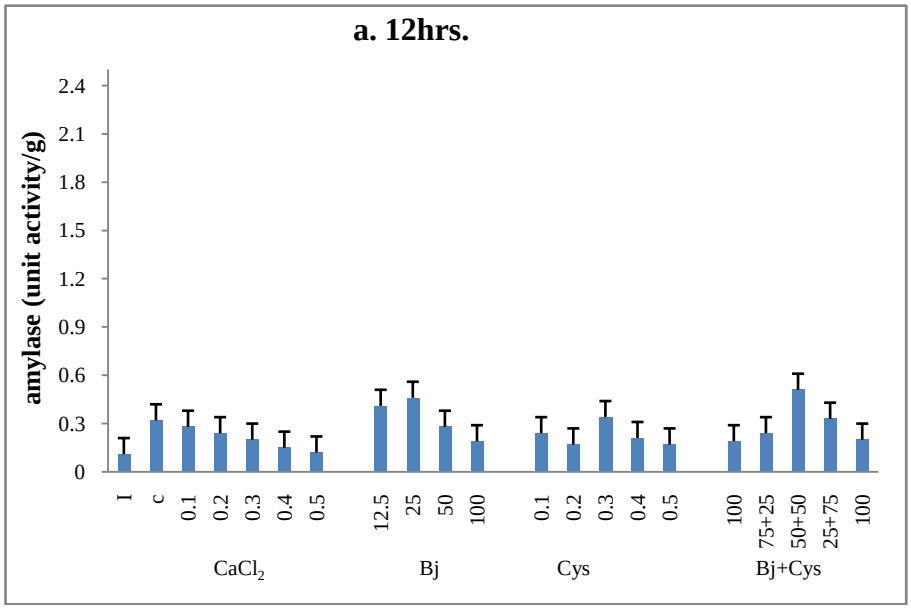
Figure 2 : Effect of different concentrations of $CaCl_2$,Bijamrita and Cyanospray on amino acid content of *Amaranthus tristis* L.(12, 24, 36 & 48 hrs)

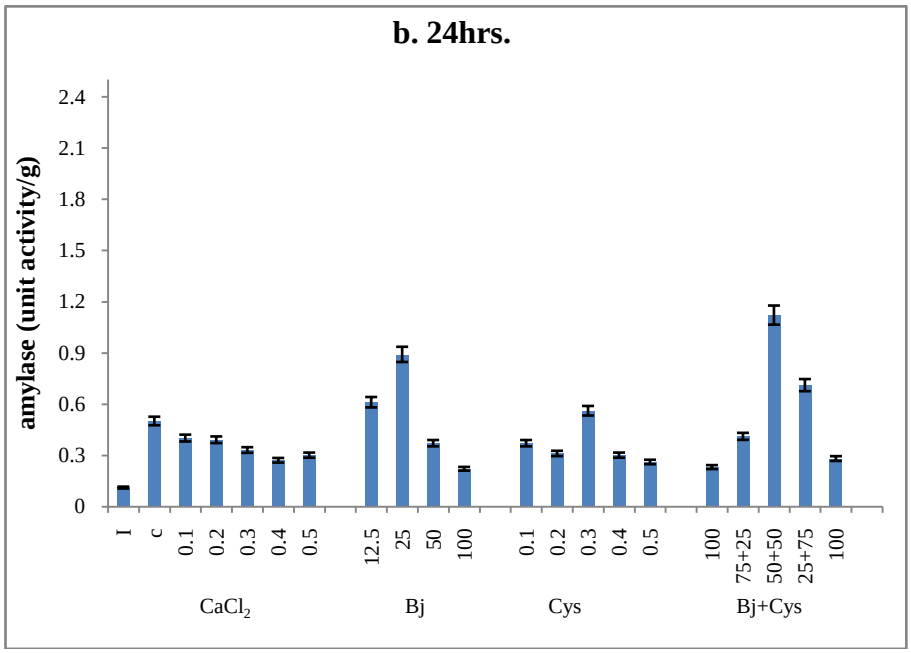
These observations were in agreement with those of (Boulter and Barber, 1963) on amino acid metabolism during germination of Viciu fuba L. (Zivile and Honorata, 2009) reported that amino acid increases during germination of Broccoli Seeds. The increase in amino acid content up to 9th day could be due to rapid hydrolysis of proteins, which resulted in release of free amino acids. The reduction in total amino acids showed rapid utilization and rapid translocation to the growing axis. Similar results were reported by (Ali Al-Heal, 1992) in Cassia senna seedlings. (Chekuboyina et al., 2012) observed that there was an increase in amino acids during germination in Ceiba pentandra seeds.

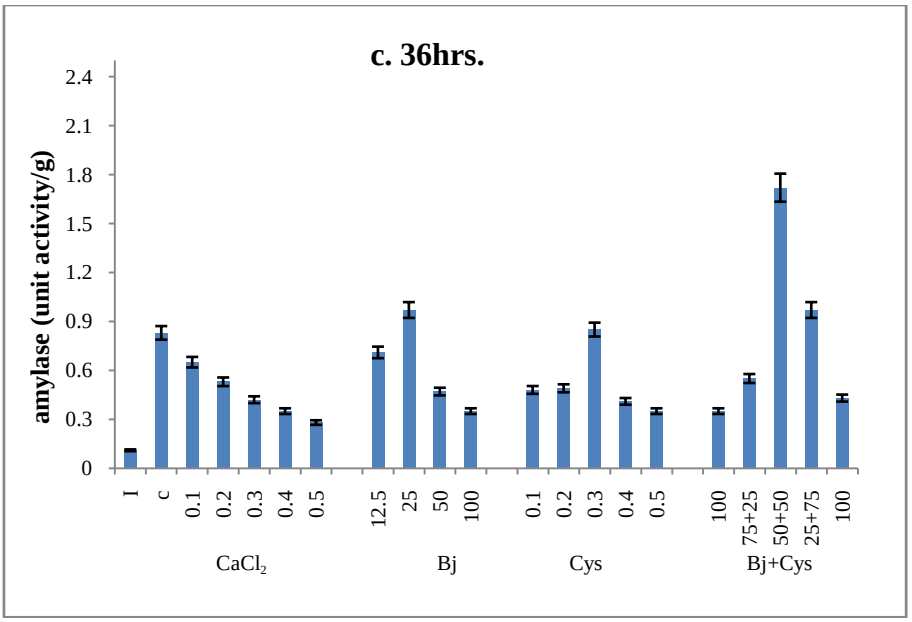
c) Assay of Amylase

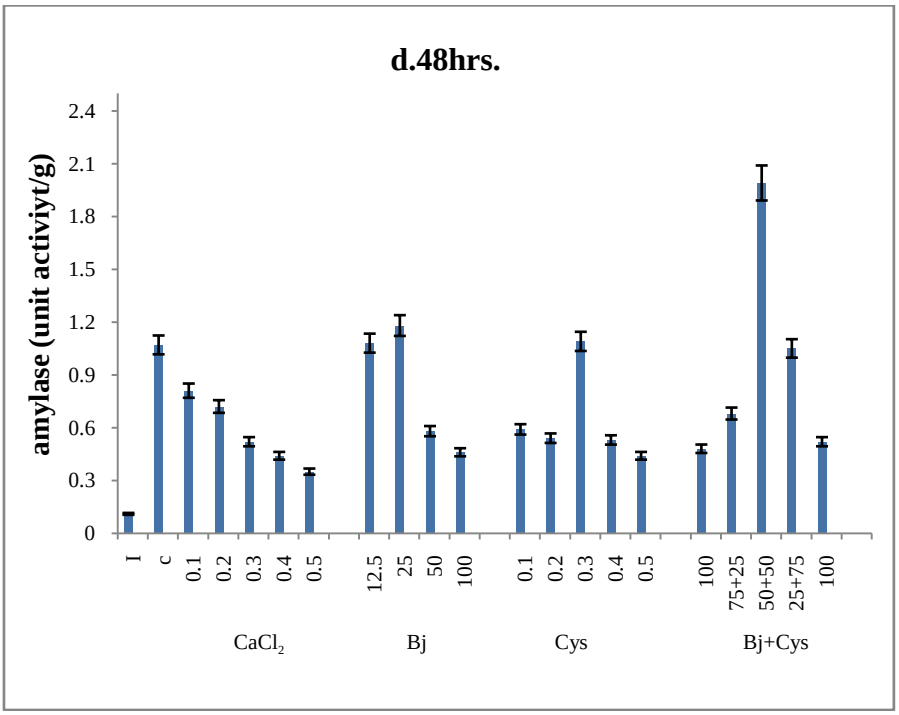
A.tristis seeds soaked with water, CaCl2, Bijamrita (25%) and cyanospray (0.3%) separately, the

activity of amylase were higher (Fig 3) at 36 and 48 hrs. But after 48 hrs the equal combination of Bijamrita (25%) and cyanospray (0.3%) showed better result than other treatments of seed germination (Fig 3.d).









-Initial, C-Control, CaCl2- Calcium Chloride, Bj- Bijamrita, Cys- Cyanospray.
(Different concentrations in percentage).

Figure 3 : Effect of different concentrations of $CaCl_2$,Bijamrita and Cyanospray on amylase content of *Amaranthus. tristis*

(Uriyo, 2001) had also reported same results in cowpea and found that germination had a high significant effect ($P<0.05$) on cowpea α-amylase activity. α -amylase activity attained a maximum level of germination for 3 days and had begun to decline on 4th day (Malleshi *et al.,* 1989). (Sumathi *et al.,* 1995) also showed improvement in α-amylase levels of horse gram, moth bean and field bean during germination. These findings agree with other reports regarding α-amylase production during germination of plant seeds other than day (Malleshi *et al.,* 1989). (Sumathi *et al.,* 1995) also (Bodin, 1995), millet (Gimbi and Kitabatake, 2002) and sorghum (Lasekan, 1996).

IV. Conclusion

The seeds of *A. tristis* were rich in proteins and their levels decreased during the germination process indicating their key role in the growth of embryonic axis.

Germination caused an increase in amino acid and amylase content in combination of 25% Bijamrita and 0.3% cyanospray (50:50) at 48hrs. This could be the optimum concentration to induce seed germination and thereafter growth of the seedlings. It also suggested that the period of the most intense mobilization of seed reserves that were stored in cotyledons occurred at germination and that the reserves were strongly reduced at the seedling growth stage.

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